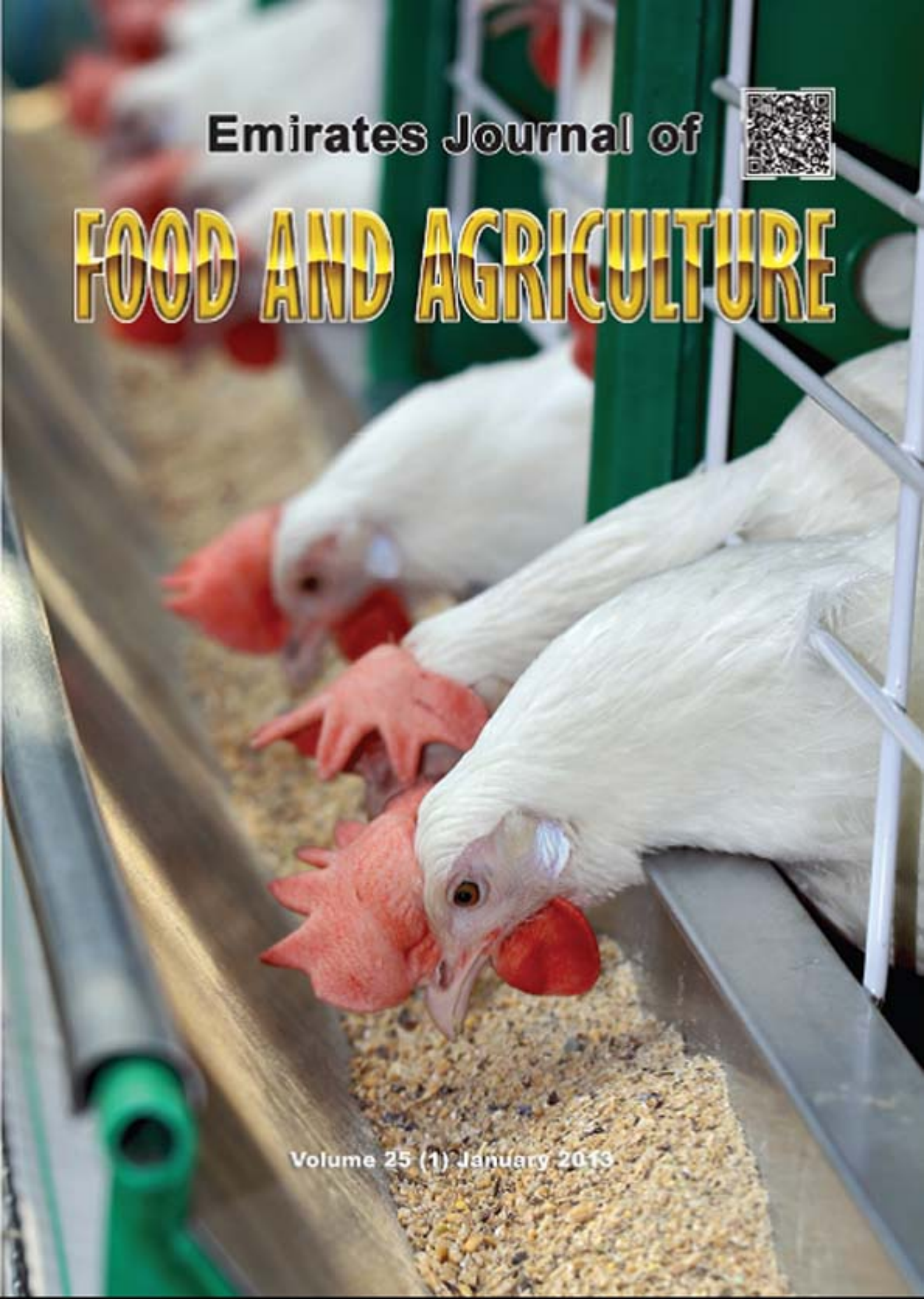

Emirates Journal of FOOD AND AGRICULTURE

Volume 25 (1) January 2013

Emir. J. Food Agric. 2013. 25 (1): 1-9
doi: 10.9755/ejfa.v25i1.9543
http://www.ejfa.info/

NUTRITION AND FOOD SCIENCE

Comparative characterization of some functional properties of flours of new plantain hybrids with the Orishele variety (*Musa* spp.) as control

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Abstract

Plantain hybrids CRBP 14, CRBP 39, FHIA 17 and FHIA 21 gives higher yields than traditional ones and have been conceived in response to the increasing plantain demand. The capability of these new hybrids to replace the traditional varieties was shown by the study of some functional characteristics of their native flours, in comparison with that of Orishele variety as control. Swelling power and solubility patterns of flours evolve in a homogeneous way for all cultivars, with however a particularity for hybrid FHIA 17, for which these phenomena occur earlier. This hybrid shows the lowest swelling values of its flour. Viscoamylograms indicate an earlier initial temperature of gelatinization (26th min at 74°C) for hybrids CRBP 14, FHIA 17 and FHIA 21 than for hybrid CRBP 39 and the control variety Orishele (30th min at 77°C). Hybrid FHIA 17 presents the highest values for viscosities at 95°C and at 50°C, as for the indices of gelation and instability. Plantain hybrids CRBP 14, CRBP 39 and FHIA 21 being characterized by technological aptitudes close to those of the control variety Orishele, they can thus replace it. Hybrid FHIA 17 would be a dessert type banana.

Key words: Plantain, *Musa* spp, Banana hybrids, Flours properties, Rheology

Introduction

Banana plants are monocotyledonous perennial and important crops in the tropical and subtropical world regions (Strosse et al., 2006). They include dessert banana, plantain and cooking bananas. Traded plantain (*Musa paradisiaca* AAB) and other cooking bananas (*Musa* ABB) are almost entirely derived from the AA·BB hybridization of *M. acuminata* (AA) and *M. balbisiana* (BB) (Stover and Simmonds, 1987; Robinson, 1996). Plantain and cooking bananas are very similar to unripe dessert bananas (*M. Cavendish* AAA) in exterior appearance, although often larger; the main differences in the former being that their flesh is starchy rather than sweet, they are used unripe and require cooking (Happi et al., 2007). Dessert bananas are consumed usually as ripe fruits; whereas ripe and unripe plantain fruits are usually consumed boiled or fried (Surga et al., 1998).

According to Ducroquet (2002) plantain (1 400 000 tones/year) is the third basic foodstuff produced in Côte d'Ivoire after yam (3 000 000 tones/year) and cassava (1 700 000 tones/year). Some of these cultivars are sensitive to diseases like the black *cercosporiosis*, which is prejudicial for a good return.

To reduce the incidence of this disease, the International Institute of Tropical Agriculture (IITA) in Nigeria, the Regional Research Center on Banana and Plantain (CRBP) in Cameroun and the *Fundaciòn Hondureña de Investigaciòn Agricola* (FHIA) in Honduras developed hybrids having a partial resistance to this phytopathology.

Tetraploid hybrid CRBP 14 of genomic AAAB group is at the same time tolerant to weevil and partially resistant to *cercosporiosis* and *fusariosis*. Its average weight of the bunch is 7.6 kg. Hybrid CRBP 39 a tetraploid plantain type (AAAB) is obtained by breeding the triploid plantain female (*Musa cv*. AAB, local variety "clear French") and the diploid synthetic hybrid male M23 (AA) banana. It presents an average bunch weight of 19.6 kg (Cohan et al., 2003). It is at the same time

Received 29 October 2011; Revised 21 March 2012; Accepted 06 May 2012; Published Online 24 November 2012

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tolerant to weevil and partially resistant to the *cercosporiosis* and the *fusariosis*.

Hybrid FHIA 17 of genomic group AAAA is tolerant to the weevil in bananas (*Cosmopolites sordidus* Germar), resistant to fungi *Mycosphaerella fijiensis* and *Mycosphearella musicola* (Molina et al., 2003). Besides, Gonzalez et al. (2003) showed its resistance not only to the black stripes disease but also to black Sigatoka disease (caused by *M. musicola* Leach) and to *fusariosis* (caused by *Fusarium oxysporum* f. sp *cubense*). Bunches of hybrid FHIA 17 have an average weight of 43.9 kg (Gonzalez et al., 2003).

Hybrid FHIA 21 (genomic group AAAB) is a plantain type resistant to Sigatoka disease and to *fusariosis*; but it is sensitive to nematodes. It has a high output (22 to 27 kg per bunch without the rachis) and the excellent quality of its fruits confers it a privileged place for consumption as in fresh (at "green yellow" maturity stage) or in cooked form (at "green" maturity stage) (Guzmán - Piedrahita et al., 2002).

Orishele is a cultivated variety of Medium False horn type; it belongs to the *Musa* genus, EUMUSA Section, AAB Species/Groups, Plantain Subgroup/subspecies. This variety is regularly used as control for agronomic trials by the researchers in the 13 African countries members of *Musa* network for West and Central Africa (MUSACO) (Anonym, 2002). Moreover, it belongs to the 13 cultivars (among 1177 currently existing accessions) stored for long period safety in cryoconservation suspension, by the World Consortium on *Musa* Genomic (INIBAP, 2004).

In addition to agronomic works, researchers in post-harvest technology did investigations on physicochemical, biochemical properties and nutritional values of many banana cultivars. Thus Orishele variety was studied by Gnakri and Kamenan (1994). Their results indicated the optimal date of harvesting it at the 72nd day after flowering. This result confirms that of Collin and Dalnic (1991).

Other works concerning the chemical composition of some varieties of Nigeria, Ghana and Côte d'Ivoire were completed by Burdon et al. (1991), Amankwah et al. (2011) and by Aboua (1991) respectively.

Coulibaly et al. (2006, 2007) extracted and characterized starches, then determined the chemical composition, the nutritional values and energy of banana hybrids CRBP 14, CRBP 39, FHIA 17 and FHIA 21 and that of Orishele. These hybrids, which can be considered as a good progress in the agronomy, have not benefits enough post-harvest investigations. With the objective of contributing to the knowledge of their technological aptitudes, our study aims to compare some flour functional properties such as swelling power, solubility patterns and viscosity with those of the Orishele variety as control. That will make it possible to determine their potential use as cooking (standard Orishele) or as dessert banana.

Material and Methods

Row material

Banana hybrids CRBP 14, CRBP 39, FHIA 17, FHIA 21 and Orishele variety were obtained from the Research Station on Fruits and Citrus Fruits of National Center of Agronomic research (CNRA), located 20 kilometers East of Abidjan (5° 38 ' N, 4° 05' W). They were all harvested at the stage of maximal maturity, when at least one ripe fruit appears on the bunch. Starting from the date of flowering, this stage corresponds to 67, 92, 78 and 82 days for the 4 hybrids CRBP 14, CRBP 39, FHIA 17 and FHIA 21 respectively. The control variety Orishele was harvested after 70 days.

Processing method of raw banana flour

The fruits of the 4 four banana hybrids (CRBP 14, CRBP 39, FHIA 17 and FHIA 21) and of the control variety Orishele were initially peeled using a stainless steel knife, washed then cut to chips. The chips were dried in a ventilated drying oven (MEMMERT, 854 Scwachbach, West Germany) at 45°C during 48 hours. The dried chips were crushed (Crushing machine model 14680, Glen Creston Ltd, Great Britain). The flower was then sieved in a 250 µm mesh sieve.

Swelling power and solubility patterns studies

These parameters were measured at treatment temperatures from 65, to 95°C using 10g db L^{-1} flour dispersion as described previously (Mestres and Rouan, 1997).

The tubes were placed in a shaking heating block and the temperature increased at 5°C/min from 50 to 95°C. After 1 hour at 95°C, the tubes were cooled at 5°C/min to 25°C and then centrifuged (Laborfuge A, Heraeus CHRIST GMBH OSTERODE, West Germany) for 15 min at 5000 x g. Supernatants and sediments were then collected and weighed (W_{su} and W_{se}, respectively). They were then dried at 105°C for 24 hours for the supernatant and 48 hours for the sediment. Their dry matter weight was then determined (D_{su} and D_{se}, respectively). The swelling power and solubility patterns were then calculated as following:

Swelling power (g/g) = $(W_{se} - D_{se}) / D_{se}$

Solubility (%) = $D_{su} / (W_{su} - D_{su})$

All measurements were performed in triplicate.

Viscosity studies of flours

The viscosity characteristics of each sample were studied using a Brabender bunchl N8025 viscoamylograph, (Duisburg, West Germany) at a fixed starch concentration of 6% db (w/w) and constant speed of 75 x g using Standard I profile as described by Garcia and Walter (1998)**.** The temperature profile employed was as follows: heating range 25 to 95°C at a rate of 5°C/min, holding at 95°C for 15 min, cooling to 50°C at the same speed and holding at 50°C for 2 min. The scale of sensitivity (torsion) used for the recording is 250 Centimorgans. The viscosity profile recorded by the Brabender reflects the peak, trough and final viscosity, pasting temperature and peak time for each sample.

Statistical analysis

The significance level of differences observed in the case of the small samples was determined with the Student t-test (Snedecor and Cochran, 1984) using the SPSS software (10th version).

Results

Evolution of flours swelling power and solubility patterns

The swelling and solubility of the 5 banana cultivars' flours according to the temperature (Figures 1 and 2) are given in the range of temperatures going from 65 to 95°C.

Concerning the flour of hybrid CRBP 14, a notable swelling (8.6 g water/g flour) is recorded only at 75°C, at this temperature hybrid CRBP 39 records the same swelling. The flour of hybrid FHIA 17 starts to swell significantly with 5.2 g water/g flour at 70°C as for hybrid FHIA 21 and the control variety Orishele. The process of notable swelling of their flour starts at 80°C with 8.3 and 8.8g water/g flour respectively.

For the 5 banana cultivars the highest swelling was reached at 95°C. It varied from 12.3 to 17.8 g water/g flour, for the flours of hybrids FHIA 17 and CRBP 14 respectively. The flour of the control variety Orishele presented at that temperature a low swelling (13.7g water/g flour).

The solubility curves (Figure 2) of the flours of the 5 banana cultivars presented in general an increasing solubility according to temperature from 4.8% (at 65°C for the control variety Orishele) to 22.3% (at 95°C for hybrid CRBP 14). The growth of this solubility was not homogeneous for all flours. It presented 3 phases:

The first phase of low solubility for the flours of all cultivars, besides hybrid FHIA 17 ranged from 65 to 70°C with solubility rates varying between 4.8% (for the control variety Orishele) and 8.7% (for hybrid CRBP 14).

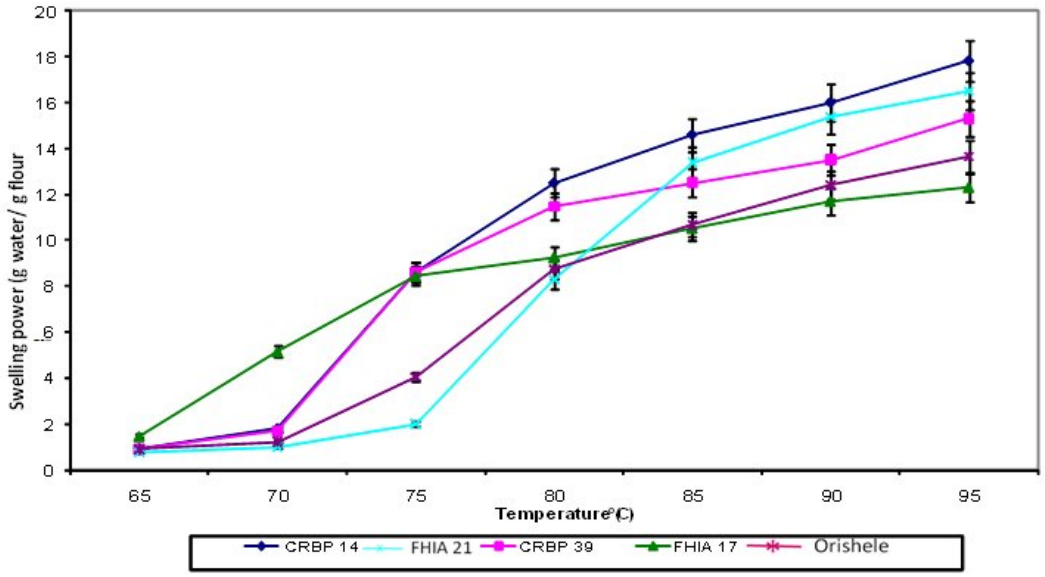

Figure 1. Flour swelling according to temperature carried out using 1% db (w/w) flour dispersion of the 5 banana cultivars (the flours was heated from 65 to 95°C in 45 min and then cooled at 5°C/min to 5°C).

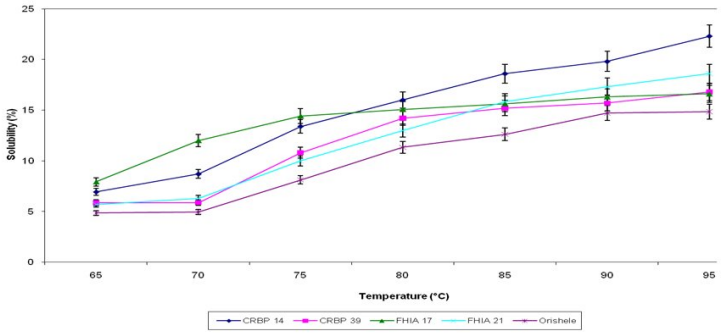

Figure 2. Flour solubility according to temperature carried out using 1% db (w/w) flour dispersion of the 5 banana cultivars (the flours was heated from 65 to 95°C in 45 min and then cooled at 5°C/min to 50°C).

The second phase was characterized by a significant solubility increase of the flours of all cultivars besides hybrid FHIA 17. It ranged from 70 to 80°C, with a flour solubility varying from 5.0 to 16.0%.

In the third phase (from 80 to 95°C), flours solubility remains low: from 13.0 (for hybrid FHIA 21) to 22.3% (for hybrid CRBP 14).

Hybrid FHIA 17 is unique with the solubility growth of its flour in 2 phases. The first one, high (from 7.9 to 12%) for temperatures ranging from 65 to 70°C; and the second one low (12.0 to 16.6%) on the last range of temperatures.

Flours pasting properties

The pasting curve of the five banana cultivars flours (Figure 3) showed some homogeneity. Flours of hybrids CRBP 14, FHIA 17, and FHIA 21 present an earlier (at the 26th minute, 74°C) increase in their viscosity, than that the flours of hybrid CRBP 39 and the control variety Orishele, whose viscosities started increasing at the 30th minute, at 77°C. For the 5 cultivars flours, the most significant increase in their viscosity was noted between the 26th and 30th minute, which corresponds to the range of temperatures between 74 and 77°C. At 77°C the highest viscosity (950 UB) and lowest viscosity (300 UB) are noted at for hybrid FHIA 21 and the control variety Orishele respectively. For all flours, maximum viscosities were reached between the 38th and 40th minute, at 95°C. Hybrid FHIA 17 and the control variety Orishele present highest (1150 UB) and lowest (770 UB) viscosity respectively.

In general, a stabilization of all viscosities was noted, between temperatures ranging from 92 to 93°C while passing by the maximum temperature 95°C.

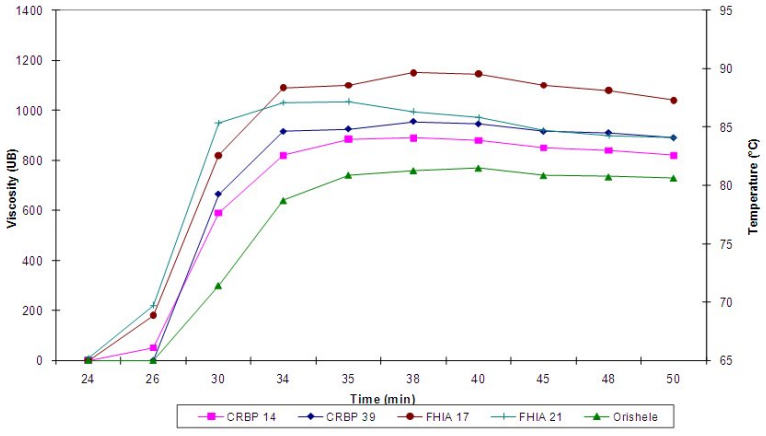

Figure 3. Viscoamylograms of the 5 banana cultivar flours according to the temperature and heating time. Flours were heated from 65 to 95°C in 45 min and then cooled at 5°C/min to 50°C).

Table 1. Viscoamylograms characteristics of the 5 banana cultivar flours.

	Tg (°C)	$V_{95\ °C\ (d)}$ (UB)	$V_{95\ °C\ (f)}$ (UB)	V_e (UB)	t_g(min)	t_m(min)	T_c(min)	$\frac{V_e - V_{95\ °C\ (f)}}{V_e}$	$\frac{V_{95\ °C\ (d)} - V_{95\ °C\ (f)}}{15\ min}$	$\frac{V_e - V_{95\ °C\ (d)}}{V_e}$
CRBP 14	74.4[a]	890±45	850±40	860±43	25±1	39±2	14±1	-0.85 ±0.1	2.7±0.3	-0.94±0.1
CRBP 39	77.2[a]	955±48	915±41	925±41	27±1	38±2	12±1	-0.74±0.1	2.7±0.2	-0.82±0.2
FHIA 17	74.5[a]	1150±60	1100±56	990±42	25±1	38±2	14±1	-0.59±0.1	3.3±0.4	-0.67±0.1
FHIA 21	74.3[a]	1035±55	920±45	955±42	24±1	34±1	10±0.5	-0.66± 0.1	7.7±0.6	-0.86±0.2
Orishele	77.1[a]	770±23	740±17	760±20	27±1	40±2	14±1	-1.06±0.1	2.0±0.1	-1.11±0.3

Values affected by the same letter in the same column are not significantly different at p = 0.05

Tg : pasting beginning temperature
Tc : Time of cooking (Time necessary to reach maximum viscosity starting from viscosity increasing)
V95 °C (d): Viscosity at 95 °C (beginning)
V95 °C (f): Viscosity after 15 min at 95 °C
Ve : Viscosity at 50 °C after cooling
tg : time of pasting beginning
tm : Time to reach maximum of viscosity
Ve-V95 °C (f) / Ve : Gelation index
V95 °C (d) –V95 °C (f) / 15 min: Viscosity drop
Ve-V95 °C (d) / Ve : Gel instability index

A drop of viscosities is recorded for all the flours, from the 45th to the 70th minute, where temperatures dropped from 95 to 50°C. The highest value (690 UB) at 50°C was noted for hybrid FHIA 17 and the lowest (360 UB) for the control variety Orishele.

Average viscosities of all cultivars varied between 519 UB (for the control variety Orishele) and 823 UB (for hybrid FHIA 17).

The starching beginning temperatures (Tg) of the flours of the 5 banana cultivars are roughly the same (Table I); they are around 75°C; the lowest (74.3°C) is noted for hybrid the FHIA 21 and the highest (77.2°C) is recorded for hybrid CRBP 39.

Hybrid FHIA 17 which has a middle starching beginning temperature (74.5°C), has with 1150 UB the highest maximum viscosity ($V_{95°C(d)}$). It is followed by hybrid FHIA 21 with 995 UB and that of hybrid CRBP 39 with 955 UB. The lowest maximum viscosity (770 UB) is recorded for the control variety Orishele.

This ranging remained the same, concerning the viscosity reached after 15 minutes at 95°C ($V_{95°C(f)}$); but an average reduction of these viscosities of 5% is noted, compared to maximum viscosities.

The viscosity drops ($V_{95°C(d)}$ - $V_{95°C(f)}$ / 15 min) noted for hybrids CRBP 14, CRBP 39 and FHIA 17 are roughly the same; it is around 3.0. The highest and the lowest viscosity drop (7.7 and 2.0) were recorded for hybrid FHIA 21 and for the control variety Orishele respectively.

After cooling at 50°C, viscosities drops (V_e) compared to maximum viscosities ($V_{95°C\ (d)}$) are noted. The highest variation (14%) was noted for hybrid FHIA 17 and the lowest (1.3%) for the control variety Orishele.

For all 5 cultivars, times of beginning of the pasting (tg) were rather close to each other (only 3 minutes between the extreme values). It was the same on the level for times necessary (MT) to obtain maximum viscosity: these times vary between 38 and 40 minutes, with however an exception at the hybrid FHIA 21, which requires only 34 minutes. This hybrid presents besides starting from the appearance of an increase in viscosity, shortest time (10 minutes) necessary to reach maximum viscosity ($V_{95°C\ (d)}$). For all the others cultivars, this time is approximately 14 minutes. The index of gelation (V_e - $V_{95°C\ (f)}$ / V_e) varies from -0.59 for hybrid FHIA 17 to -1.06 for the control variety Orishele. All of the instability index (V_e - $V_{95°C\ (d)}$ / V_e) of the 5 banana cultivars flours gel is negative and very close to 0. They vary from -0.67 for hybrid FHIA 17 to -1.11 for the control variety Orishele.

Discussion

The representation of swelling rate according to heating temperature going from 65 to 95°C shows a hydration speed for the 5 banana cultivars flours. All the curves present a regular increase, with a homogeneous slope starting from 85°C and the maximum swelling at 95°C going form 12.3 and 17.8 g water/g flour for hybrids FHIA 17 and CRBP 14 respectively. Our results corroborate those of Coulibaly et al. (2006), which indicated for starches of the same banana cultivars, a regular increase of swelling with a homogeneous slope starting at 85°C. These authors present maximum swellings at 95°C between 16.1 and 35.6 g water/g starch recorded for the same hybrids (FHIA 17 and CRBP 14). These results were also in the same order of those obtained by Delahaye et al. (2008) with unirape plantain flour in chamber dryer conditions. Dadier et al. (1998) noted a regular swelling for the flours of 2 sweet

potato (*Ipomea batatas*) varieties (red skin white flesh and yellow skin yellow flesh) between 65°C and 95°C. By considering initial swelling temperatures, Tetchi et al. (2007) noted on starches of various products, different ranges of swellings temperatures (from 55°C to 65°C, from 65°C to 75°C and from 75°C to 85°C). The swellings values recorded at 95°C by Dadier et al. (1998) located around 20 g water/g flour, were higher than the present one (12.3 and 17.8 g water/g flour). The present results however were close on one hand to 16 g water/g starch obtained by Amani et al. (2004) at 95°C on ginger starch, and on the other hand to 12.9g water/g starch obtained by Tetchi et al. (2007) on corn starch.

Solubility according to temperature (from 4.8 to 22.3%) of the tested bananas flours are quit the same with those (from 0.9 to 28.2%) obtained by Coulibaly et al. (2006) on the bananas starches. At low temperatures (from 65 to 75°C), average flours solubility (4.8%) are higher than those of starches (0.9%). That was due to the low solubility of starches because of homogeneous cohesion forces, which maintain the starch granule matrix (Reyes et al., 1983). Flours solubility rates of the tested bananas correspond to those obtained by Dadier et al. (1998), who recorded on flours of 2 sweet potato (*Ipomoea batatas*) (red skin yellow flesh and yellow skin yellow flesh) solubility rates varying between 5% (at 65°C) and 23% (at 80%). These values corroborate our results (4.5% and 16%). On the other hand for temperatures ranging from 80 to 95°C, our values (from 11.3 to 22.3%), remain lower than those given by Dadier et al. (1998) on sweet potato flours (from 23 and 34%), and those (from 23.0 to 31.7%) obtained by Amani et al. (1997) on cocoyam flours.

There is proportionality between solubility and swelling power of the flours (Figure 4).

The curves of tendencies are lines whose equations are $y = ax + B$, with $0.7909 \leq a \leq 0.8825$ and $4.4545 \leq B \leq 7.1486$. The value of R^2 for the different lines varies from 0.9532 for hybrid FHIA 17 to 0.9902 for hybrid CRBP 14. These values very close to 1 indicate a very close correlation between the solubility and the swelling power of the flours. This result confirms former work of Coulibaly et al. (2006), who on the same banana starches noted a very strong correlation between solubility and swelling power, with values of R^2 varying between 0.9837 and 1.

The study of solubility according to swelling power generally showed a linear relation between the two phenomena. Thus, a swelling increase was accompanied by a high solubility. This phenomenon was observed by several authors (Amani et al, 1997; Delpeuch and Favier, 1980; bertolini et al., 2010).

The pasting curve of the 5 banana flours has the same shape. For hybrids CRBP 14, FHIA 17 and FHIA 21 the first notable viscosity increase was recorded after 26 minutes at 74°C. For hybrid CRBP 39 and the control variety Orishele the significant viscosity variation occurs at the 30th minute at 77°C. These 2 temperatures (74 and 77°C) are those of the beginning of flour gelatinization of these 2 banana groups. The flours swelling evolution according to temperature corresponds to the initial pasting temperature (Tg) of these flours. Comparatively, low swellings were observed at 70°C for the flours of hybrid CRBP 39 and the control variety Orishele; that is coherent with their relatively high initial pasting temperature. Temperature of 78°C was reported for the beginning of gelatinization for *Xanthosoma sagittifolium* (red and white *cv.*) native starches Amani et al. (1997).

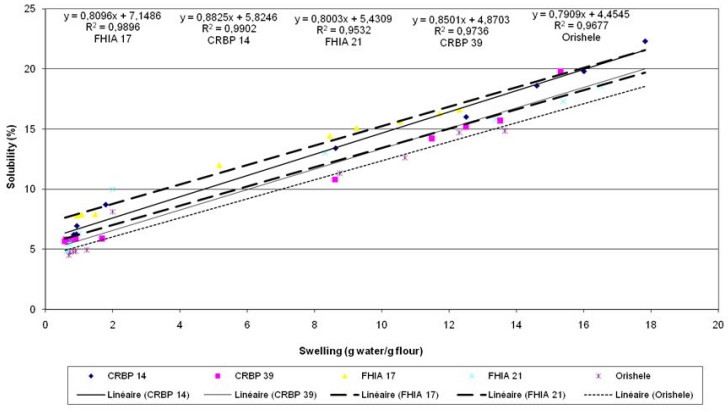

Figure 4. Straight lines of flour solubilisation according to flour swelling of the 5 banana cultivars.

On the starches of various products, Tetchi et al. (2007) noted initial temperatures of gelatinization varying between 70°C for rice and 90°C for ginger. Gnakri (1993) indicated the gelatinization beginning at 74°C for the starch of the Orishele variety. For the starch of the banana *Musa paradisiaca*, Núñez-Santiago et al. (2004) noted the beginning of gelatinization at 75°C. The viscosity increase at the beginning of gelatinization is due to starch solubility between 70 and 95°C, corresponding to starch paste formation. It follows a second phase during which the gel formation occurs (Leach, 1965; Duprat et al., 1980; Adunni and Olaposi, 2010).

After cooling at 50°C, the gelation index (V_e – $V_{95°C\ (f)}$ / V_e) of all flours remain negative (from -1.06 for the control variety Orishele to -0.59 for hybrid FHIA 17). Therefore, they do not have any reconstitution capacity of their starch macromolecular network. According to Leach (1965) and Duprat et al. (1980), when the temperature is lowered gradually until 50°C, there is retrogradation by a partial reconstitution of the macromolecular network of the starch in the flour. This reconstitution determines the final consistency of the product after cooling. It is a function of amylopectin and amylose concentrations in the flour. Instability index of gel (varying from -1.11 to -0.67) are very close to 0. That implies good gel stability. This result confirms those of Eggleston et al. (1992) that indicated a great stability and a negligible retrogradation on several plantain (*Musa* spp., AAB group) starches. The gel instability Index of hybrids CRBP 14, CRBP 36 and FHIA 21 (-0.94; -0.82 and -0. 86 respectively) are very close to that of the control variety Orishele (-1.11); that augurs them an identical aptitude of cooking in water. Hybrid FHIA 17 with -0.67 as instability index, produces the least stable gel; the control variety Orishele with -1.11 has the most stable gel. The flour of hybrid FHIA 17 has moreover the highest viscosities at 95°C as well as at 50°C (after cooling).

Conclusion

This study was carried out to determine some functional properties of banana hybrids CRBP 14, CRBP 39, FHIA 17 and FHIA 21, in comparison with those of the control variety Orishele.

The swelling power and the solubility patterns of flours according to temperature progressed homogeneously for all bananas, with however a particularity for hybrid FHIA 17, for which these phenomena occurred earlier.

The flours viscoamylogramms indicate an earlier initial pasting temperature for hybrids CRBP 14, FHIA 17 and FHIA 21, than that of hybrid CRBP 39 and the control variety Orishele. Hybrid FHIA 17 shows the most stable gel and the control variety Orishele, least stable.

Hybrid FHIA 21 is characterized by the shortest times for beginning of empesage and for cooking. The control variety Orishele presented average values for all characteristics.

Looking at the results, the banana hybrids CRBP 14, CRBP 39 and FHIA 21 are similar to the control variety Orishele. Moreover, Hybrid FHIA 17 is significantly different from the other hybrids and the control variety Orishele. These result made CRBP 14, CRBP 39 and FHIA 21 able to replace the control variety Orishele, as regards the culinary preparations in vegetable oil. And Hybrid FHIA 17 could be classified among dessert bananas.

Complementary work in vivo should determine nutritional qualities of these new banana hybrids, as well as their aptitudes for the preparation of local dishes of high consumption such as "Foutou"(water boiled and pounded bananas), "Foufou" (water boiled and crushed bananas) and "Concondé" (banana stew). That will open ways for popularizing these hybrids in farming system at rural level.

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Emir. J. Food Agric. 2013. 25 (1): 10-19
doi: 10.9755/ejfa.v25i1.9368
http://www.ejfa.info/

NUTRITION AND FOOD SCIENCE

Potential of nanotechnology in functional foods

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Abstract

Food nanotechnology is a relatively recent area which has opened up a whole universe of new applications in food industry. Some of these applications include; improved taste, flavor, color, texture and consistency of foodstuffs, increased absorption and bioavailability of nutraceuticals and health supplements, development of food antimicrobials, new food packaging materials with improved mechanical barrier and antimicrobial properties, nanosensors for traceability and monitoring the condition of food during transport and storage, encapsulation of food components or additives. Smart delivery of nutrients, bioseparation of proteins, rapid sampling of biological and chemical contaminants and nanoencapsulation of nutraceuticals are few more emerging areas of nanotechnology for food industry. Nanotechnology holds great promise to provide benefits not just within food products but also around food products. There is an urgent need for regulatory systems capable of managing any risks associated with nanofoods and the use of nanotechnology in food industry. In this review, applications of nanotechnology in functional food with special attention to related regulatory issues are discussed.

Key words: Functional Foods, Food Industry, Nanotechnology, Nano-food

Introduction

The term 'nano' is derived from the Greek word for dwarf (Sangamithra and Thirupathi, 2009). The term "Nanotechnology" was first used in 1974 by the late Norio Taniguchi and concepts were given by Richard Feynman in 1959 (Warad and Dutta, 2005). Nanoscience is defined as the study of phenomena and the manipulation of materials at the atomic, molecular and macromolecular scales, where the properties differ from those at a larger scale (Mannino and Scampicchio, 2007).

The National Nanotechnology Initiative (2006) has defined nanotechnology as the understanding and control of matter at dimensions of roughly 1 - 100 nm, where unique phenomena enable novel applications; encompassing nanoscale science, engineering and technology, nanotechnology involves imaging, measuring, modeling, and manipulating matter at this length scale (Chen et al., 2006a). In 2006, Food and Drug Administration (FDA) defined nanomaterials as "particles with dimensions less than micrometer scale that exhibit unique properties" (Miller and Senjen, 2008).

Nanotechnology in agricultural and food industries was first addressed by a United States Department of Agriculture (USDA) roadmap published in September 2003 (Joseph and Morrison, 2006). A range of nanotechniques and materials are being developed in an attempt to assert greater control over food character traits, to enhance processing functionalities, such as flavour, texture, speed of processing, heat tolerance, shelf life, traceability, safety, the bioavailability of nutrients and cost effective food analysis major focus is on functional foods as they offer the ability to control and manipulate properties of substances close to molecular level (Chaudhry et al., 2008; Scrinis and Lyons, 2007; Weiss et al., 2006).

Nanotechnology in the food industry

The term "nanofood" describes the food which has been cultivated, produced, processed or packaged using nanotechnology techniques or tools, or to which manufactured nanomaterials have been added (Morris, 2007). Nanofood has, in fact, been part of food processing for centuries, since

Received 13 October 2011; Revised 13 July 2012; Accepted 01 August 2012; Published Online 24 November 2012

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many food structures naturally exist at the nanoscale (Shekhon, 2010). The applications of nanotechnology for the food sector fall into the following main categories:

- Where nano-sized, nano-encapsulated or engineered nanoparticle additives have been used;
- Where food ingredients have been processed or formulated to form nanostructures;
- Where nanomaterials have been incorporated to develop improved, active, or intelligent materials for food packaging or in food contact materials or surfaces;
- Where nanotechnology-based devices and materials have been used, e.g. for nanofiltration, water treatment;
- Where nanosensors have been used for food safety and traceability and contaminant detection (Chaudhry et al., 2008; McCall, 2007).

Nanofood market

The worldwide sales of nanotechnology products in the food and beverage packaging sector increased from US$ 150 million in 2002 to US$ 860 million in 2004 and are expected to reach to US$ 20.4 billion by 2010 (Helmut Kaiser Consultancy, 2004). The consulting firm Cientifica, has estimated the then (2006) food applications of nanotechnologies at around $410 million (food processing US$100 million, food ingredients US$100 million and food packaging US$210 million). According to the report, the existing applications are mainly for improved food packaging, with some applications for delivery systems for nutraceuticals. The report estimated that by 2012 the overall market value would reach US$5.8 billion (food processing US$1303 million, food ingredients US$1475 million, food safety US$97 million and food packaging US$2.93 billion) (Cientifica, 2006).

More than 200 companies are actively involved in research and development (Asadi and Mousavi, 2006). USA is the leader followed by Japan and China (Helmut Kaiser Consultancy, 2004). There is a large potential for growth of the food sector in developing countries. Today, many of the world's leading food companies including H.J. Heinz, Nestlé, Hershey, Unilever, and Kraft are investing heavily in nanotechnology research and development (Joseph and Morrison, 2006; Kuzma and VerHage, 2006; Shelke, 2006; Miller and Senjen, 2008).

Kraft's global 'Nanotek Research Consortium' of 15 universities and national research centre, reflects a corporate strategy to lead developments for a nano food future (http://www.ifst.org/science_technology_resources/for_food_professionals/information_ statements / index/#). As of March 2006, over 200 ''nano'' consumer products are currently available, and about 59% and 9% of the products are categorized as ''Health and Fitness'' and ''Food and Beverage'' products, respectively (Chau et al., 2007).

Processes for nanomaterial production

The two approaches to attain nanomaterials are *top-down approach* and *bottom-up approach* (Table 1). The ''topdown'' approach involves physically machining materials to nanometre size range by employing processes such as grinding, milling, etching and lithography. For example, a high water-binding capacity wheat flour of fine size can be obtained by dry-milling technology. By contrast, self-assembly and self-organization are concepts derived from biology that have inspired a bottom-up food nanotechnology. Bottom-up techniques build or grow larger structures atom by atom or molecule by molecule. These techniques include chemical synthesis, self-assembly and positional assembly (Acosta, 2008; Sanguansri and Augustin, 2006; Sozer and Kokini, 2009; Meetoo, 2011).

Table 1. Range of sizes of nanomaterials in the food sector.

Structures	Diameter or length (nm)
DNA	12
Glucose	21–75
Liposome	30–10000
LDH	40–300
Amylopectin	44–200
Casein micelle	60–100
PLA nanosphere	100–300
Zein	200
Cubosome	500
Nanosensors	<1000

Owing to the greater surface area of nanoparticles per mass unit, they are expected to be more biologically active than larger sized particles of the same chemical composition. This offers several perspectives for functional food applications. Nanoparticles can, for instance, be used as bioactive compounds in functional foods (Sozer and Kokini, 2009).

Natural self-assembled nanostructures

Many natural foods contain nanoscale components and their properties are determined by

their structure. These have been eaten safely for generations. In fact, some of most important nutrients of raw materials *i.e.,* proteins, starches and fats, undergo structural changes at the nanometer and micrometer scales during normal food processing (Morris and Parker, 2008). Milk proteins (for example, native beta-lactoglobulin, which is about 3.6 nm in length) can undergo denaturation (via pressure, heat, pH, *etc*.) and the denatured components reassemble to form larger structures, like fibrils or aggregates, which in turn can be assembled to form even larger gel networks (*e.g.,* yogurt). Self-assembled nanotubes from hydrolyzed milk protein α-lactalbumin, a potential new carrier for nanoencapsulation of nutrients, supplements, and pharmaceuticals, have been reported (Bugusu et al., 2009). Casein micelles may be useful as nanovehicles for entrapment, protection and delivery of sensitive hydrophobic nutraceuticals within other food products (Semo et al., 2007).

A cow's udder is an excellent example of a nano device synthesizing, assembling, and dispensing proteins and fat into an aqueous phase, where they later become building blocks for protein structures. Processes such as homogenization and fine milling causes microstructural changes in food. Homogenized milk has a nanostructure of 100 nm sized droplets in it. The dairy industry utilizes three basic micro sized and nanosized structures (casein micelles, fat globules, whey proteins) to build all sorts of emulsions (butter), foams (ice cream and whipped cream), complex liquids (milk), plastic solids (cheese), and gel networks (yogurt). In fact, dairy technology is not just a microtechnology but also a nanotechnology, and it has existed for a long time. Research into naturally occurring nanostructures in foods is mainly designed to improve the functional behavior of the food (Shekhon, 2010).

Nanoparticulate delivery systems

Nanoencapsulation

Nanoencapsulation is incorporation of ingredients in small vesicles or walled material with nano (or submicron) sizes (Surassmo et al., 2010). These nanomaterials offer several advantages such as, delivery vehicle for lipid soluble ingredients, protection from degradation during processing or in GIT, controlled site specific release, compatible with other food constituents, greater residence time and greater absorption (Chen et al., 2006a, b; Weiss et al., 2006). The protection of bioactive compounds, such as vitamins, antioxidants, proteins, and lipids as well as carbohydrates may be achieved using this technique for the production of functional foods with enhanced functionality and stability. Nanoencapsulation can make significant savings for formulators, as it can reduce the amount of active ingredients needed (Huang et al., 2009). Table 2 lists the products reported with such nanomaterials.

Nanoencapsulation of probiotics

According to FAO/World health organization (WHO) (2002), probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". They can be incorporated in fermented milk, yoghurts, cheese, puddings, fruit based drinks etc. Nanoencapsulation used to develop designer probiotic bacterial preparations that could be delivered to certain parts of the gastro-intestinal tract where they interact with specific receptors. These nanoencapsulated designer probiotic bacterial preparations may act as *de novo* vaccines, with the capability of modulating immune responses (Vidhyalakshmi et al., 2009).

Nanosized self-assembled liquid structures

Nanosized self-assembled liquid structures are used as vehicles for targeted nutraceuticals. The vehicles are expanded micelles in the size of <30 nm and used in "clear" beverages without phase separation. Potential applications include lycopene, beta-carotene, omega- 3 fatty acids, phytosterols, and isoflavones (Garti and Aserin, 2007).

Applications in food processing

Nanofrying

The US based Oilfresh Corporation has marketed a new nanoceramic product which reduces oil use in restaurants and fast food shops by half because of its large surface area (Joseph and Morrison, 2006; Pehanich, 2006).

Novel foods

A Hungarian company has developed an ice gel for soft drinks or ice-cream containing CO_2 bubbles of 1-10 nm in diameter for effervescence (http://files.nanobio-raise.org/ Downloads/ Nano technology-and-Food-fullweb.pdf). Nanotech spray is available in which Nanodroplets 87 nm are used to enhance the uptake of vitamin B_{12} and other supplements for use in foods (Bouwmeester, 2007). Kraft Foods and NanoteK consortium have plans to incorporate the electronic tongue (which is chemical change based biosensor) into foods to release accurately controlled amounts of the suitable molecules for the customized tailor-foods (Shelke, 2006; Sozer and Kokini, 2009).

Table 2. Nanoencapsulates in food products.

Nanoencapsulates	Products	References
Nanoemulsions - Oil in water emulsion, usually 50-500nm	β-Carotene; α-Tocopherol;Nanoemulsion based ice-cream (Nestle, Unilever)	Chu et al., 2007; Ribeiro et al., 2008; Tan et al., 2005; Yuan et al., 2008; Joseph and Morrison, 2006; Miller and Senjen, 2008
Biopolymeric Nanoparticles - Dense matrix network of sub-100nm in which active molecules are dispersed throughout	β- lactoglobulin; Nanoceuticals™ (RBC Life Sciences®); Nano Calcium/ Magnesium (Mag-I-Cal.com USA); nano-selenium-enriched Nanotea (Shenzhen Become Industry)	Bouwmeester, 2007; Chaudhry et al., 2008; Chen and Subirade, 2005: Sabliov and Astete, 2008; Shelke, 2006; Miller and Senjen, 2008; Zimet and Livney, 2009
Nanocapsules - Vesicles in which oil or liq. ingredients are confined within polymeric membrane of (20-100nm)	Nanocapsules of Capsium, oleoresin, eugenol, lysozymes, vitamins, phytosterols; "Tip top" up bread with nano fish oil (Nu-Mega), Kraft foods-personalized flavours and colours	Choi et al., 2009 a,b; Jafari et al., 2008; Joseph and Morrison, 2006; McCall, 2007; Semo et al., 2007; Shelke, 2006; Surassmo et al., 2010, Türk and Lietzow, 2004; Zhong et al., 2009
Nanospheres - Solid colloidal particles enclosed by polymer matrix with several phases in suspension	Omega 3 fatty acids, whey protein nanospheres; Citral flavour (Key Lime Formulations); Chocola Chocolate chewing gum with nanococoa (Olala)	Shelke, 2006; Subirade and Chen, 2008; Chen et al., 2006b
Nanoliposomes- Polymeric aggregates of lipid bilayers esp. phospholipids such as egg or soy	Lactoferrin, nisin, phosvitin, enzymes, vitamins, antioxidants, coenzyme Q10	Bouwmeester, 2007; Chen et al., 2006a; Liu and Park, 2010; Malheiros et al., 2010; Raman and Danino, 2008; Teixeira et al., 2008
Nanocochleates -Cigar shaped multilayered structure with spiral solid lipid bilayer	Bioral™ for nutrients (BioDelivery systems)	Chaudhry et al., 2008; Morris, 2007; Sangamithra and Thirupathi, 2009; Joseph and Morrison, 2006; Miller and Senjen, 2008
Nanoclusters	Slim shake chocolate, Nanoceuticals (RBC Life sciences); Nanococoa (Royal Body care)	Bouwmeester, 2007; Chaudhry et al., 2008; Morris, 2007; Shelke, 2006; Miller and Senjen, 2008.
Nanomicelles- Sub 100nm spherical particles formed spontaneously upon surfactant addition after Critical micelle conc. has reached	Limonene, carvacrol, lutein, eugenol, Omega 3 fatty acids, whey proteins, essential oils; Lycopene (BASF Germany), NutiNano™(Solgar, USA); Novosol (Aqanova®) for nutrients; Canola Activa Oil (Sheman Ind.)	Bouwmeester, 2007; Chaudhry et al., 2008; Chen et al., 2006a; Garti and Aserin, 2007; Gaysinsky et al., 2008; Joseph and Morrison, 2006; Losso, 2007; Zhang et al., 2009; Miller and Senjen, 2008;
Nanotube- 20 nm in diameter and several μm in length	α-lactoglobulin as gelling agent, delivery of nutrients and flavours	Bikker and Kruif, 2006; Ipsen and Otte, 2007
Nanofibers- 100nm fibers from polymer solutions	Vitamins, β-carotene, eugenol, antioxidants, flavors	Arecchi, 2009; Fernandez, 2009

Nanofiltration

Nano Filtration separating materials of less than 0.001 microns (10 angstroms) in size and rejects divalent and multivalent ions (Cuartas-Uribe et al., 2007; Sangamithra and Thirupathi, 2009). It is an alternative to Reverse Osmosis as it is cost effective (Sangamithra and Thirupathi, 2009). It has application in desalination, milk, whey and juice filtration, demineralization, colour removal, concentration of products, waste water treatment and water purification (Kaul, 2005; Doyle, 2006; Cuartas-Uribe et al., 2007; Duke et al., 2008; Tiwari et al., 2008; Diaz-Reinoso et al., 2009; Sangamithra and Thirupathi, 2009).

Food processing equipments

Nanotechnology-based translucent insulant coatings called Nansulate PT has been reported for dairy processing tanks and pipes for reduced costs and extended life (Pehanich, 2006; Baurah and Dutta, 2009).

Immobilization of enzymes

Nanoporous media, nanofibers, carbon nanotubes, magnetic nanoparticles are used for enzyme immobilization (Mao et al., 2006; Kim et al., 2008; Kosseva et al., 2009). Immobilization of lipase on nanofibers and magnetic nanoparticles for

soybean oil hydrolysis have been reported (Li and Wu, 2009; Lee et al., 2009; Wang et al., 2009).

Application in food packaging

Nano packaging applications as Food Contact Materials (FCMs) are anticipated to grow from a $66 million business in 2003, to over $360 million by 2008 (Scrinis and Lyons, 2007). Applications for food contact materials (FCMs) using nanotechnology is as follow:

- FCMs incorporating nanomaterials to improve packaging properties (flexibility, gas barrier properties, temperature/moisture stability, light and flame resistant, transparency, mechanical stability).
- ‘‘Active’’ FCMs that incorporate nanoparticles with antimicrobial or oxygen scavenging properties.
- ‘‘Intelligent’’or “smart” food packaging incorporating nanosensors for sensing and signaling of microbial and biochemical changes, release of antimicrobials, antioxidants, enzymes, flavours and nutraceuticals to extend shelf-life
- Biodegradable polymer–nanomaterial composites by introduction of inorganic particles, such as clay, into the biopolymeric matrix and can also be controlled with surfactants that are used for the modification of layered silicate (Sozer and Kokini, 2009; Chaudhry et al, 2008; Miller and Sejnon, 2008; Joseph and Morrison, 2006; Doyle, 2006; Lopez- Rubio et al. 2006; Brody, 2007).

Improved packaging

A variety of nanoparticle reinforced polymers, also termed as ‘‘nanocomposites’’ have been developed, which typically contain up to 5% w/w nanoparticles with clay nanoparticle composites with improved barrier properties (80-90% reduction) for the manufacture of bottles for beer, edible oils and carbonated drinks and films (Chaudhry et al, 2008; Meech, 2007; Brody, 2007). Example of available nanoclay composites include Imperm® (from Nanocor® Inc.), Aegis® X (Honeywell), Durethan® KU2-2601 (Bayer AG), Plastic Technology, Miller Brewing Co. (USA), Hite Brewery Co. Nanoparticle composites include DuPont Light Stabilizer 210’, Durethan®, Bayer’shaving (Scrinis and Lyons, 2007; Chaudhry et al, 2008; Sozer and Kokini, 2009; Giner et al, 2009 Meech, 2007; Miller and Sejnon, 2008). United States Food and Drug Administration (USFDA) has approved the use of nanocomposite in contact with foods (Sozer and Kokini, 2009).

Active packaging

Nano silver, Nano magnesium oxide, nano copper oxide, nano titanium dioxide and carbon nanotubes are also predicted for future use in antimicrobial food packaging (Doyle et al, 2006, Miller and Sejnon, 2008; Chaudhry et al, 2008). Kodak company is developing antimicrobial packaging for food products that will be commercially available in 2005 and ‘active packaging,’ which absorbs oxygen (Asadi and Mousavi, 2006). Other companies include FresherLonger™ Miracle Food Storage Containers’’ and ‘‘Fresher Longer™ Plastic Storage Bags’’ from Sharper Image® USA, ‘‘Nano Silver Food Containers’’ from A-DO Korea, and ‘‘Nano Silver Baby Milk Bottle’’ from Baby Dream® Co. Ltd. (South Korea). Oxygen scavenging packaging using enzymes between poly ethylene films have also been developed (Lopez et al, 2006). An active packaging application could also be designed to stop microbial growth once the package is opened by the consumer and rewrapped with an active-film portion of the package (Brody, 2007). Zinc oxide quantum dots were utilized as a powder, bound in a polystyrene film (ZnO-PS), or suspended in a poly vinyl prolidone gel (ZnO-PVP) as antimicrobial packaging against *Listeria monocytogenes, Salmonella enteritidis, and Escherichia coli* O157:H7 (Sun et al, 2009).

Smart/ Intelligent packaging

BioMerieux have developed a multi-detection test – Food Expert ID for nano surveillance response to food scares. Nano-scale radio frequency identification tags (RFid) have been developed to track containers or individual food items (Burrah and Dutta, 2009) and are being used in retailing chains (Joseph and Morriosn, 2006; Asadi and Mousavi, 2006). The nanotech company pSiNutria are also developing nano-based tracking technologies, including an ingestible BioSilicon which could be placed in foods for monitoring purposes and pathogen detection, but could also be eaten by consumers (Scrinis and Lyons, 2007; Miller and Sejnon, 2008). US company Oxonica Inc has developed nano barcodes (20-500 nm in diameter and 0.04-15 mm in length) to be used for individual items or pellets, which must be read with a modified microscope for anti-counterfeiting purposes (Miller and Sejnon, 2008; Warad and Dutta, 2005). Engineered nanosensors are being developed by Kraft along with Rutgers University (U.S.) with in packages to change colour to warn the consumer if a food is beginning to spoil, or has

been contaminated by pathogens using electronic 'noses' and 'tongues' to 'taste' or 'smell' scents and flavours (Joseph and Morrison, 2006, Asadi and Mousavi, 2006; Scrinis and Lyons, 2007; Sozer and Kokini, 2009). Nestlé, British Airways, MonoPrix Supermarkets are using chemical nanosensors that can detect colour change (Pehanich, 2006).

Edible nano coatings

A nanolaminate consists of 2 or more layers of material with nanometer dimensions that are physically or chemically bonded to each other (1-100 nm per layer, usually 5 nm) could be used to encapsulate various hydrophilic, amphiphilic, or lipophilic substances, active functional agents such as antimicrobials, antibrowning agents, antioxidants, enzymes, flavors, and colors with improved moisture and gas barrier properties and can be created entirely from food-grade ingredients (proteins, polysaccharides, lipids). These functional agents would increase the shelf life and quality of coated foods such as meats, cheese, fruit and vegetables, confectionery, bakery goods and fast food (Wiess et al, 2006). United States Company Sono-Tek Corp. announced in early 2007 that it has developed an edible antibacterial nano coating which can be applied directly to bakery goods; it is currently testing the process with its clients (Miller and Sejnon, 2008).

Nanosensors in food safety and analysis

A nanosensor is a device consisting of an electronic data processing part and a sensing layer or part, which can translate a signal such as light, or the presence of an organic substance or gas into an electronic signal structured at the nanometre scale (http://files.nanobio-raise.org/Downloads/ Nanotechnology-and-Food-fullweb.pdf).

Array biosensors, Nanoparticle based sensors, Nanocantilevers, Nano-test-strips, Nanoparticles in solution; electronic noses etc. have been used as nanosensors to detect pathogens, toxins, chemicals and analysis in foods (Doyle, 2006; Bhattacharya et al., 2007; Baurah and Dutta, 2009; Naja et al., 2010; Sozer and Kokini, 2009; Tang et al., 2009; Valdes et al., 2009). EU-funded BioFinger project has developed portable biosensor Bio-Nano and MicroElectroMechanical Systems (BioMEMS) using nanocantilevers for detection of biological entities, chemicals and toxins (Joseph and Morrison, 2006).

Toxicology and safety aspect of nanoparticles

The nanomaterials incorporation into foods presents a whole new array of risks for the public, workers in the food industry and farmers because: (1) Are more chemically reactive than larger particles; (2) have greater access to our bodies than larger particles (3) Enhanced toxicity due to greater bioavailability (4) can compromise our immune system response and (5) may have longer term pathological effects (Hoet et al., 2004; Miller and Senjen, 2008).

They translocate to skin, brain, liver etc and cause oxidative damage in cells. Reports on nano-particle uptake by endothelial cells, pulmonary epithelium, intestinal epithelium, alveolar macrophages, other macrophages, nerve cells and other cells are available and may also be associated with rising levels of immune system dysfunction and inflammations of the gastrointestinal tract, including Crohn's disease (Chaudhry et al., 2008). They can translocate from lungs to blood, Central Nervous System (CNS) through nerve cells and have been implicated in Parkinson's and Alzheimer's disease (Chau et al., 2007; Miller and Sejnon, 2008). They can also cause oxidative damage to the cells (Chaudhry et al., 2008). Long term exposure can cause acute toxic response such as lesions in kidneys and liver, granulomas, cancers, clots etc. Nanoparticles can also be taken up by the broken or damaged skin or even by brain cells (Miller and Sejnon, 2008). Particles smaller than 70 nm can enter cell nuclei and even cause impairment of DNA replication and transcription (Chaudhry et al., 2008).

Regulations

The European Union regulations for food and food packaging have recommended that for the introduction of new nanotechnology, specific safety standards and testing procedures are required (Halliday, 2007). In the United States, nanofoods and most of the food packaging are regulated by the USFDA (Badgley et al., 2007), while in Australia, nanofood additives and ingredients are regulated by Food Standards Australia and New Zealand (FSANZ), under the Food Standards Code (Bowman and Hodge, 2006). In India food safety regulations are introduced but not adequate for the monitoring safety of nanoparticles.

Existing laws are inadequate to assess risks posed by nano based foods and packaging because: (1) Toxicity risks remain very poorly understood (because of their unique properties); (2) are not assessed as new chemicals according to many regulations (3) Current exposure and safety methods are not suitable for nanomaterials and (4) many safety assessments use confidential industry studies (Chaudhry et al., 2008; Miller and Senjen,

2008). Up to now, there is no international regulation of nanotechnology or nanoproducts.

Conclusion

The use of nanotechnology to manufacture of processed foods with enhanced processing, health and packaging functionalities - flavour, texture, shelf-life, transportability, reduced costs and nutritional traits will facilitate the expansion of the range, quality and quantity of processed foods, and to thereby meet the contemporary demands for both 'health' and 'convenience'. It has enabled the development of food safety and food quality aspects. But in the end, regulatory considerations (including safety/toxicology and environmental impact), economics, and consumer acceptance of nanotechnology will ultimately dictate its success in food applications.

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Emir. J. Food Agric. 2013. 25 (1): 20-29
doi: 10.9755/ejfa.v25i1.15376
http://www.ejfa.info/

PLANT SCIENCE

Wheat Landraces: A mini review

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Abstract

Traditional farmers planted diverse assemblages of wheat landraces to lower the risk of failure and increase food security because they had limited capacity to control the spatially heterogeneous and temporally unpredictable environments. This practice led to the development of landrace meta-populations of wheat and the emergence of farmers' seed systems through which they accessed and exchanged diverse genetic material. During the last ~50 years, the introduction of high-yielding wheat varieties into, and the structural changes in wheat farming systems in developing countries, led to the loss of genetic diversity and fragmentation of meta-population structures of wheat landraces from large parts of the Fertile Crescent, the center of origin and diversity of wheat landraces. However, the persistence cultivation of some wheat landraces attests to their continued value to farmers, or to their competitive agronomic or nutritional advantage relative to modern varieties. For farmers to continue to grow, select, and manage local wheat landraces, and to reverse the fragmentation of their meta-populations, especially in their center of diversity, and allow evolutionary processes that mold landrace diversity to continue, their value should be raised to approximate or exceed the social value of high-yielding wheat varieties. This review provides information on wheat domestication and the origin of wheat landraces; their dynamic on-farm conservation and utilization in improving modern wheat cultivars and reversing the genetic erosion of wheat genetic diversity.

Key words: Wheat, Landrace, Diversity, Fertile Crescent

Introduction

Wheat domestication was responsible for the increase in human population by enabling humans to produce food in large quantities, thereby contributing to the emergence of the human civilization (Zohary and Hopf, 2000). The domestication of wild emmer (*Triticum dicoccoides*), the progenitor of all cultivated wheats (Feldman and Kislev, 2007), was one of the key events during the emergence of agriculture in Southwest Asia, and was the prerequisites for the evolution of tetraploid durum and hexaploid bread wheat. However, the domestication of wild emmer in the Fertile Crescent and the subsequence breeding of domesticated durum and bread wheat drastically narrowed their genetic diversity (Dvorak et al., 1998). Upon domestication, it was estimated that initial diversity was reduced by 84% in durum wheat and by 69% in bread wheat. Historically, traditional farmers planted diverse assemblages of wheat genotypes (i.e., landraces) to lower the risk of failure and increase food security because they had limited capacity to control the spatially heterogeneous and temporally unpredictable environment (Jaradat, 2006). This practice led to the development of landrace meta-populations of wheat and the emergence of farmers' seed systems through which they accessed and exchanged diverse genetic material. A meta-population structure, defined as a group of subpopulations interconnected by gene-flow and seed exchange among farmers, villages and eco-geographical regions, favors a dynamic evolution of diversity.

Wheat landraces are composed of traditional crop varieties developed by farmers through years of natural and human selection and are adapted to local environmental conditions and management practices (Zeven, 1999). As distinct plant populations, landraces are named and maintained by traditional farmers to meet their social, economic, cultural, and environmental needs. They are alternately called farmers' varieties or folk varieties (Belay et al., 1995) to indicate the innovative role of farmer communities in their development and maintenance.

Received 14 March 2012; Revised 07 May 2012; Accepted 06 June 2012; Published Online 24 November 2012

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The genetic structure of wheat landraces is an evolutionary approach to survival and performance (Brown, 2000), especially under arid and semi-arid growing conditions (Jaradat, 1992b). The combined effects of natural and human selection have led to architecture of genotypes representing different combinations of traits, such as growth habit, cold, heat or drought tolerance, early growth vigor, time to heading and maturity, seed filling duration, and quality traits (Masood et al., 2005). As a result, wheat landraces developed into complex, variable, genetically dynamic and diverse populations, in equilibrium with both biotic and abiotic stresses in their environment. Throughout their history, farmers subjected wheat landraces to strong selection pressures; therefore, wheat landraces developed multi-locus structures as a result of selection, genetic drift, or fragmentation of their populations (Brown, 2000). These structures predominantly are retained through selection, isolation, lack of migration, and restrictions on outcrossing and genetic recombination. Little has been done to understand the genetic structure of wheat landraces and the inter-specific diversity available in the subsistence agro-ecosystems they still dominate in parts of the Old World (Altieri and Merrick, 1987).

Durum (Jaradat, 2006) and bread wheat landraces (Ehdaie and Waines, 1989a;b) have been largely replaced, in their centers of diversity, by monocultures of pure genotypes. This genetic erosion resulted in significant loss of valuable genetic diversity for adaptation to low or organic inputs and for resistance to biotic and abiotic stresses. The pure genotypes of high yielding wheat varieties may not have the wide adaptation and the diverse genetic background already present in landraces that they replaced (Ali Deb et al., 1992). The development of new varieties from landrace populations is a viable strategy to improve landrace yield and yield stability, especially under stress and future climate change conditions. Due to their high nutritive value, modern wheat cultivars are superior to other cereals in providing energy and high quality protein for billions of people around the world. However, the need is urgent to increase the yield potential and improve nutritive quality (Koshgoftarmanesh et al., 2010) and tolerance to biotic and abiotic stresses (Ehdaie and Waines, 1989b) of cultivated wheat in view of climate change, rising demand for healthy wheat products, and the increasingly alarming loss of its wild genepool. Wheat landraces are valuable sources to broaden the genetic base of cultivated wheat. The development of new varieties from landrace populations is a viable strategy to improve landrace yield and yield stability, especially under stress and future climate change conditions (Witcomb et al., 1996); also, these landraces harbor genes and gene complexes for quality traits (Zencirci and Karagoz, 2005), tolerance to biotic and abiotic stresses, and adaptation under a wide range of low-input and organic farming systems (Jaradat, 2006).

Wheat Domestication

The domestication of wheat around 10,000 years ago marked a dramatic turn in the development and evolution of human civilization (Willcox, 1998), as it enabled the transition from a hunter-gatherer and nomadic pastoral society to a more sedentary agrarian one. Two of the most important traits in the evolution of wheat and other cultivated grasses constitute the domestication syndrome. These were:

1. An increase in grain size, which was associated with successful germination and growth of seedlings in cultivated fields, and
2. The development of non-shattering seed, which prevented natural seed dispersal and allowed humans to harvest and collect the seed with optimal timing. Size and shape of the wheat grain are independently inherited traits and the domestication process resulted in a switch from production of a relatively small grain with a long, thin shape to a more uniform larger grain with a short, wide shape.

The complex history of domesticated wheat evolution (Feldman and Sears, 1981), suggested that various traits arose independently at different stages. Grain size, for example, may have increased early in domestication through changes in grain width and length, followed at later stages by further modifications in grain shape. Later during the course of wheat evolution, the decrease in phenotypic diversity in grain morphology in modern commercial wheat is attributed to a relatively recent and severe bottleneck that may have occurred either during the transition from hulled to the modern free-threshing wheat, or even more recently as a result of modern breeding programs.

Molecular genetics and archaeological data have allowed the reconstruction of possible domestication scenarios leading to the development of landraces, old and then modern cultivars (Dvorak et al., 1998; Willcox, 1998). For diploid (2x) *einkorn* and tetraploid *turgidum* (hard) wheat (4x), a single domestication event has likely occurred in the Karadagh Mountains, Turkey. Following a cross between tetraploid *turgidum* and

diploid goat grass, the resultant hexaploid (6x) bread wheat was disseminated around the Caucasian region, then around the Old World. These events, although resulted in wheat domestication, created genetic bottlenecks (Hammer et al., 1996), which excluded potentially adaptive alleles. More recently, the same phenomenon was repeated upon the development of high yielding wheat varieties at the expense of losing much of the diversity in wheat landraces and old cultivars. A significant decrease of genetic diversity has been observed related to the replacement of bread wheat landraces by high yielding cultivars which appear to be associated with loss of some quality traits such as protein content and glutenins quality (Distefeld et al., 2007).

Throughout most of last ~10,000 years, farmers have been behind the development and conservation of wheat genetic diversity (Zeven, 2000). The landraces and old cultivars they developed can be considered as evolutionary links between wild emmer wheat, the wild progenitor of all domesticated wheats, and advanced wheat cultivars. The extinction of traditional farming systems, erosion, or even the aging and exodus of rural population, and more recently, environmental degradation (Mercer and Peralis, 2010), have led to the extinction of many local landraces. As a consequence, during the last century most of the unique cereal biodiversity has disappeared and the information regarding landraces and traditional cultivars is presently very scarce. Several authorities (Hammer et al., 1996; Witcombe et al., 1996) estimated that almost 75% of the genetic diversity of crop plants was lost in the last century. This erosion of these genetic resources results in a severe threat to the world's long-term food security. Although often neglected, the urgent need to preserve and utilize landrace genetic resources as a safeguard against an unpredictable future is evident.

Origin of Wheat Landraces

Thousands of years of cultivation aided by natural and human selection have resulted in the evolution of immense diversity of genotypes in the predominantly self-pollinated wheat species. Throughout their evolutionary history, wheat crops have been shaped and molded mainly by farmers to meet diverse end uses (Zeven, 2000), cultural practices, and to respond to changing socio-economic and growing conditions (Cox and Wood, 1999). A number of socio-cultural factors, food traditions, and agro-ecological environments favored the cultivation and utilization of diverse wheat genetic resources, including primitive or hulled (e.g., *Triticum monococcum, T. dicoccum, T. spelta*), and free-threshing wheat species (e.g., *T. durum, T. polonicum, T. compactum, T. aestivum*), constituting what is now known as landraces. Each wheat species or landrace has particular significance in the food culture, as a source of daily diet, and of food and drink for special occasions (Dhillon et al., 2004). Wheat landraces generally have both private and public values. Landraces constitute a private good to the farmers who grow them; whereas, to institutions engaged in their conservation and improvement, landraces constitute a public good and a source of useful genetic material.

Traditional management of wheat landraces contributed more to the conservation of a general level of diversity than to the conservation of genetically stable and distinct populations. Therefore, a wheat landrace is not necessarily a genetically and phenotypically stable, distinct, and uniform unit. Its diversity is linked to the diversity of the material sown in its immediate geographical vicinity, and to the level and frequency of short- and long-distance seed exchange among farmers (Morris and Heisey, 1998). Wheat landraces embody not only diverse alleles and genotypes, but also evolutionary processes such as gene flow between different populations, mainly via seed exchange and local knowledge systems such as folk taxonomies and information about selection for specific quality attributes or for heterogeneous environments (Zeven, 2000). The complexity of the population structure of wheat landraces may arise from the number of different homozygotes and the occurrence and frequency of heterozygotes in populations. Therefore, characterization of the population structure of wheat landraces is critical to identify and correctly interpret the association between their functional and molecular diversity (Brown, 2000). Such information is essential to utilize landraces as donors of traits in wheat breeding, to define the areas of adaptation of different landraces, to identify priority areas for on-farm conservation, and to understand the genetic consequences of the interaction between climate change, growing environments and farmers' practices (Motzo and Giunta, 2007).

As compared to modern wheat varieties, landraces, with relatively higher biomass, may not invest in larger root dry mass, but rather in increased partitioning of root mass to deeper soil profiles, increased ability to extract moisture from those depths, and higher transpiration efficiency. In addition, their increased concentration of soluble

carbohydrates in the stem shortly after anthesis ensures adequate translocation of assimilates to the developing grains. Therefore, early maturity, with some yield penalty, is a valuable trait that can be derived from wheat landraces to combat the typically-encountered season-end drought in rainfed wheat production regions (Ayed et al., 2010). Facultative growth habit is a unique characteristic of some wheat landraces; it provides flexibility of sowing either in the fall as a winter crop or, after the failure of the crop to overwinter, again in the spring. Under growing conditions with limited nitrogen availability, wheat landraces and old varieties with a taller growth habit and lower harvest index absorb and translocate more nitrogen into the grain than modern varieties (Geneç et al., 2005), presumably due to greater pre-anthesis uptake and an increased buffering capacity in genotypes with high vegetative biomass. Therefore, appropriately selected landraces with well-developed root systems could be a source of variation for nutrient uptake, and the improvement of seed quality. Mineral content in modern wheat cultivars has significantly decreased, including copper, iron, magnesium, manganese, phosphorus, selenium, and zinc (Geneç et al., 2005; Distefeld et al., 2007). High levels of these nutrients can be found in landraces and old low-yielding varieties. Because wheat landraces have been developed mostly in environments with low nutrients availability, they represent a source of variation for selection of varieties adapted to cropping systems with low fertilizer input. Compared to the cost associated with the formation of new roots, arbuscular mycorrhiza may considerably increase the active absorbing root surface with minor cost to the wheat plant, thus enhancing the uptake of phosphorus, in particular, and other macro- and micro-nutrients, in general (Distefeld et al., 2007; Koshgoftarmanesh et al., 2010).

Only a limited number of studies have focused on quality aspects of organic wheat production (Onduru et al., 2002). This trait is of particular concern to organic farmers and consumers since protein content in organic cereals tend to be lower due to the difficulty and costs of foliar application of inorganic nitrogen fertilizers applied later in the growing season. A higher protein content and quality without the need for late-season nitrogen inputs are therefore major breeding objectives. However new varieties should be particularly suitable for whole-meal bread making and artisan baking processes, combining sensory and nutritional qualities (e.g., increased micro-nutrients) as the consumers of organic bread expect highest organoleptic quality (Zencirci and Karagoz, 2005). Farm households allocate resources for production of favorite or preferred landraces, expecting benefits to accrue from their subsequent consumption or sale in local markets (Brush and Meng, 1998). Farmers continue to grow a wheat species or landrace and maintain it if it meets their production and consumption needs. Therefore, direct use values, particularly the quality traits that farmers consider as valuable for consumption are indicators of private value. Socio-cultural values motivate farmers to retain some preferred landraces on the farm, and they appreciate the special organoleptic qualities and multiple uses of these landraces, despite the availability of improved wheat varieties in their locality (Zencirci and Karagoz, 2005). Landraces, especially those having multiple home uses, are more likely to be maintained for the foreseeable future. Therefore, home use values can serve as a strong incentive to encourage continued cultivation and utilization of wheat landrace by farm households (Frison et al., 2011). Nevertheless, research will be necessary to verify some of the claims made by farmers concerning peculiar culinary qualities of their preferred wheat landraces. These include, for example, better nutritional value of the grain or its products, and superior medicinal or aesthetic value of local drinks made from wheat landraces.

Conservation and Utilization of Wheat Landraces

Clearly much landrace germplasm has been collected during the 1970-1990 era and is being conserved across the world mostly in long-term national and international genebanks (Frizon et al., 2011). However, a small portion of this diversity is being conserved and used on-farm where it continues to evolve (Brush and Meng, 1998). Both of these conservation methods have its merits and limitations. On-farm conservation is the sustainable management of genetic diversity of locally-developed traditional crop cultivars and landraces along with associated wild and weedy species or forms within traditional agricultural systems. This conservation strategy provides a natural laboratory for evolution to continue and helps a gradual buildup of traits imparting adaptation to specific eco-geographical regions and those matching the requirements of farmers, local communities and populations to continue. Several authorities indicated that the need for on-farm conservation of landraces is one of the most important recent questions in plant genetic resources management

(Le Boulch et al., 1994; Kebebew et al., 2001). Farmers continue to grow and maintain a wheat landrace if it meets their production and consumption needs. The total cost and benefit of landraces to farmer households are central to their on-farm conservation and continued utilization. Farmers maintain crop landraces if these are valued either for economic, cultural, social, or even ecological reasons. Therefore, direct use values, particularly the quality traits that farmers regard as valuable for consumption are considered to be proxy indicators of private value of a landrace (Brush and Meng, 1998).

Research results indicated that the likelihood of wheat landraces to be conserved on the farm increases when the markets for their derived products are expanded through improved consumer access to information on recipes, nutritive and cultural values. Therefore, local knowledge of landrace diversity, when documented through interaction with farmers and linked to food traditions, local practices and social norms, is vital for on-farm conservation and would increase their competitive advantage if farmers have other alternative options. For example, socio-cultural values and culinary attributes motivated farmers in central Ethiopia to conserve a durum wheat landrace on their farms; they appreciate its peculiar organoleptic qualities and multiple uses, including 14 dishes and two drinks, despite the availability of several improved durum wheat varieties in their locality (Kebebew et al., 2001). Moreover, hundreds of farmers who accessed the landrace through reintroduction program expressed their appreciation and future commitment to growing and conserving it on the farm. This example strongly indicated that farmers in a community collectively can sustain more crop and landrace diversity than individual farmers, thus meeting overall conservation needs and objectives (i.e., private and public values of a landrace). A renewed interest in and increased demand by farmers to grow this durum wheat landrace and the promotion of landrace-derived products generated income, created green jobs for local communities, and supported on-farm conservation of the landrace. Along with economic benefits, on-farm conservation and utilization of such wheat landraces is also linked to peoples' cultural, social and ritual values. However, for individual farmers, private values of a landrace are the main motivating factors for growing landraces as a source of income and a means of survival. Therefore, *ex situ* conservation in a genebank may be the only practical option to conserve landraces having low private but high public value (Le Boulch et al., 1994).

Seed Saving and Exchange Systems

Global biodiversity and plant genetic diversity constitute the raw materials humans rely on for food, fiber, forage, fuel, medicine and many industrial products. The National Plant Germplasm System (NPGS), a publically-funded germplasm conservation system, is a part of the Agricultural Research Service (ARS) of USDA and is responsible for collecting, conserving, characterization, evaluation, distribution, and exchange of a rich and diverse genetic resources collection containing about 500,000 accessions (GRIN, 2011; www.ars-grin.gov). The wheat genetic resources are housed at the National Small Grains Collection (NSGC), which is part of NPGS-ARS. The NSGC is an active germplasm collection that maintains seed samples representing global diversity of the small grains including wheat (*Triticum*, see list of species and subspecies below), barley (*Hordeum*), oat (*Avena*), rice (*Oryza*), rye (*Secale*), triticale (*X Triticosecale*), and various wild relatives (including *Aegilops*). Germplasm is maintained in the form of seed or live plants, representing current, obsolete and primitive crop varieties and landraces, wild and weedy relatives of crop species, and wild species collected from around the world.

The Germplasm Resources Information Network database (GRIN, 2011; www.ars-grin.gov) describes collection holdings of the NPGS. The NSGC's *Triticum* spp. collection currently includes the following species and subspecies (Table 1) that can be accessed through the active links: *Triticum aestivum* subsp. *aestivum* (44,975 accessions), *T. aestivum* subsp. *compactum* (113 accessions), *T. aestivum* subsp. *macha* (31 accessions), *T. aestivum* subsp. *spelta* (1,295 accessions), *T. aestivum* subsp. *sphaerococcum* (32 accessions), *T. ispahanicum* (7 accessions) , *T. monococcum* subsp. *aegilopoides* (918 accessions), *T. monococcum* subsp. *monococcum* (210 accessions), *T. timopheevii* subsp. *armeniacum* (269 accessions), *T. timopheevii* subsp. *timopheevii* (42 accessions), *T. turgidum* subsp. *carthlicum* (95 accessions), *T. turgidum* subsp. *dicoccoides* (921 accessions), *T. turgidum* subsp. *dicoccon* (620 accessions), *T. turgidum* subsp. *durum* (8,403 accessions), *T. turgidum* subsp. *paleocolchicum* (4 accessions), *T. turgidum* subsp. *polonicum* (80 accessions), *T. turgidum* subsp. *turanicum* (107 accessions), *T. turgidum* subsp. *turgidum* (457

accessions), *T. urartu* (210 accessions), *T. vavilovii* (3 accessions), and *T. zhukovskyi* (7 accessions).

Table 1. Wheat species, sub-species and number of accessions available at the Genetic Resources Information Netwrok, USA.
(GRIN, 2011; www.ars-grin.gov).

Wheat species	Sub-species	Number
Triticum aestivum	*aestivum*	44,975
T. aestivum	*compactum*	113
T. aestivum	*macha*	31
T. aestivum	*spelta*	1,295
T. aestivum	*sphaerococcum*	32
T. ispahanicum		7
T. monococcum	*aegilopoides*	918
T. monococcum	*monococcum*	210
T. timopheevii	*armeniacum*	269
T. timopheevii	*timopheevii*	42
T. turgidum	*carthlicum*	95
T. turgidum	*dicoccoides*	921
T. turgidum	*dicoccon*	620
T. turgidum	*durum*	8,403
T. turgidum	*paleocolchicum*	4
T. turgidum	*polonicum*	80
T. turgidum	*turanicum*	107
T. turgidum	*turgidum*	457
T. urartu		210
T. vavilovii		3
T. zhukovskyi		7

The GRIN database contains passport data, information which describes where and when an accession was collected, donated or developed. Crop-specific descriptor lists have been developed for most crops to provide a means of comparing accessions within a collection based upon standardized morphological, phonological, physiological, biochemical and molecular traits, as well as disease and insect tolerance or resistance. The GRIN system provides information on the availability and amount of seed that can be freely distributed to scientists and farmers in the US and around the world. However, the typically small amount of seed that farmers can obtain from the GRIN system may not satisfy their immediate needs. Moreover, there is a substantial time lag implicated in restoring landrace diversity on the farm from the typically small seed quantities conserved and distributed by genebanks to be immediately used by farmers. Therefore, the continued production of landraces through on-farm conservation ensures timely availability of quality seed, and allows for the dynamic evolution of landraces under diverse agro-ecosystem.

Low-input and organic family farms require reliable sources of producible seed that are well adapted to local farming practices, local food needs, and market conditions. Small farmers who are not able to reproduce and save their own seed on the farm may suffer financially from dependency on the purchase of high-cost commercial seed. Local availability and access to high quality seed are key factors in the efforts to sustain on-farm conservation of wheat landraces. Therefore, to address gaps in the supply side and enhance local seed security, farmers need to restore and strengthen informal seed networks and community seed banks, and seek technical advisory services from traditional seed experts (Qualset et al., 1997).

Small-scale family farms traditionally save seed of heirloom or local varieties in order to sustain harvests and conserve well-adapted traditional crop varieties. Seed saving can contribute to lower supply costs, more diversified goods, improved human nutrition, and farm self-sufficiency. On-farm seed saving by small farmers is essential in conserving global agricultural biodiversity (Witcombe et al., 1996), in general, and crop diversity, in particular. Recently, however, this effort has been undermined by corporate consolidation of seed markets and the contentious concerns about seed types, sources, and availability. Commercial and large-scale seed industries are constantly developing seeds that represent genetically uniform, high-yielding, and increasingly genetically modified crop varieties. These seed types are of little or no value to organic and low-input farmers; they are usually designed for use in large-scale mechanized farming, and sometimes are packaged with chemical inputs. As modern industrialized farming extends over the global agricultural landscape, the seed industry has become both more technically specialized and increasingly controlled by large corporate firms. The new seed technologies may pose serious and complex economic risks to small farmers (Rijal, 2010); they can become dependent on expensive improved seed varieties and brands that are marketed along with complementary agrochemical packages. In addition, some commercial cultivars may not meet local dietary needs (e.g., gluten-sensitive patients) or market demand (e.g., semolina for traditional confectionery products).

Recreating and structuring local seed systems to simulate a source-sink meta-population model is a first step towards restoring the fragmented meta-population structures of wheat landraces. Through this model, stakeholders can (Almekinders et al., 1994; Zeven, 1999):

1. Identify the unit of analysis (e.g., the farmer as a decision maker and agent of conservation, the field or parcel representing a particular habitat, the landrace, or a seed lot),
2. Incorporate variation among farmers in their practices, knowledge and gender,
3. Quantify patterns of seed exchange among farmers and their impact on the biology parameters of landrace population,
4. Identify the limiting factors that determine distribution and range of a landrace; and,
5. Define the minimum area needed to create a dynamic equilibrium between "colonization" and "extinction" of a landrace meta-population.

The goal of this type of participatory endeavor is empowering the farmers by supporting the formation of groups capable of assessing their own needs and addressing them either directly or through demands on publically-funded research organizations. Unfortunately, not every smallholder farmer can easily select and save adequate supplies of seed from each harvest. The ability and choice of each farmer to save seed depends on many factors, including availability of labor, technical training and skills in seed conservation, food needs, farm income, and market conditions. Moreover, low income family farms may have limited technical capability and facilities to produce and properly store seed lots, and thus can face risks in conserving and sustaining reliable and high-quality seed supplies for their planting needs.

Traditional farmers periodically resort to replacing seed of their old varieties and landraces with seed from other farmers to combat what they consider as “seed degradation.” This “inexplicable” seed replacement may have its origins in farmers’ belief that homegrown seed degenerates after several generations of re-sowing under the same environmental and edaphic conditions and management practices (Zeven, 1999). Moreover, some farmers are convinced that traditional maintenance breeding may not result in higher yield; therefore, they felt that seed replacement was a better method to maintain productive capacity of their crops. Arguably, seed replacement and avoidance of traditional maintenance breeding by farmers could be attributed to the existing, but mostly unsuspected, negative association between yield potential of the landrace and the competitive ability of individual plants within its genetically heterogeneous populations. As seed of many old varieties and landraces disappear across the world and sales of modern improved seed varieties increase exponentially, more low-income farmers may face difficult choices about the type and source of the seeds they utilize (Baniya et al., 2000).

Landraces and the Future of Wheat Diversity

Durum and bread wheat landraces have been largely replaced, in their centers of diversity by monocultures of pure genotypes. This genetic erosion resulted in significant loss of valuable genetic diversity for quality traits and resistance or tolerance to biotic and abiotic stresses; whereas, the pure wheat genotypes do not have the wide adaptation and the diverse genetic background already present in landraces. Diversity of wheat landrace populations, when structured to build spatial and temporal heterogeneity into cropping systems will enhance resilience to abiotic and biotic stresses. Other resilience sources will include more robust genetic resistances and biochemical response mechanisms derived from landrace genotypes (Bonman et al., 2007).

Climate change is expected to differentially affect components of complex biological interactions in modern and traditional wheat production systems. Wheat yield and quality will be affected by climate change directly, and indirectly, through diseases (e.g., stem and leaf rusts) that themselves will change but remain important (Newton et al., 2011). These effects will be difficult to dissect and model as their mechanistic bases are generally not well-understood. The manner with which wheat landraces and their populations in and outside their centers of diversity might respond to climate change will determine their continued productivity, utility, and survival. Phenotypic plasticity, evolution, and gene flow, although each presents its own uncertainty, are possible avenues for surviving shifts in biotic and abiotic conditions caused by climate change. Whether there will be constraints on evolution in response to the abiotic and biotic stresses caused by climate change, modern wheat, but not landrace adaptation may not keep up enough to maintain fitness (i.e., seed production). Wheat plants will probably respond through shifts in morphology (e.g., tillering capacity, leaf area index, green leaf area duration), phenology (e.g., days to anthesis, days to maturity, duration of seed filling period), or development (e.g., rate of leaf emergence based on available growing degree days), which may help maintain fitness. However, phenotypic plasticity and gene flow (mainly through seed exchange) of landraces may not produce fully adapted phenotypes or the necessary genetic variation to combat climate change. Declining yields of landrace populations due to expected climate change would cause great

concern to farming families and threatens their livelihoods. In their attempt to maintain yields, farmers would consider changing seed sources and discarding their adapted landrace populations (Zeven, 1999). This could result in the loss of certain landrace populations, entire landraces, or, in extreme cases, whole minor wheat species.

The development of new varieties from wheat landrace populations is a practical strategy to improve yield and yield stability, especially under stress and future climate change conditions. Further enhanced productivity and stability can be achieved through practicing continuous selection within landraces across the marginal production environments, to exploit the constantly released useful adaptive variation (Ehdaie and Waines, 1989b). Non-breeding approaches to create demand for landrace products to promote on-farm dynamic conservation and sustainable utilization of wheat landraces include:

1. Raising public awareness regarding current and future value of landraces,
2. Diversity fairs to allow for the exchange of landrace materials and associated indigenous knowledge,
3. Visits among farmers in different localities to share seeds and experiences,
4. Diversity contests to reward farmers who keep special varieties and or conserve the highest diversity, and
5. Recipe development and niche market creation for landrace products. Together, these activities are expected to complement each other and contribute positively towards sustaining on-farm conservation and landrace diversity for the foreseeable future.

Landraces, as an important genetic resource, have been included in international treaties and national decrees that protect and enhance their use in their local environments. However, legislation is needed to make it possible to market landraces as diversified genetic materials. National and international legislation was designed primarily to protect trade and return royalty income to expensively-funded plant breeding programs; as landraces become more attractive to use in local food production and sustainability, legislation changes are needed to facilitate this trend and to promote exportation and exchange of landrace diversity and encourage their use (Jaradat, 1992a; Joshi and Witcomb, 2003).

Conclusions

Wheat landraces are better adapted than modern cultivars to changing climate conditions and to stress environments due to their population genetic structure, buffering capacity, and a combination of morpho-physiological traits conferring adaptability to stress environments. However, their low yield, as compared to high yielding varieties, could be attributed to their genetic heterogeneity and to inter-plant competition which can be eliminated when a landrace is converted into desirable homozygous genotypes. For farmers to continue to grow, select, and manage local wheat landraces, and to reverse the fragmentation of their meta-populations and eventual genetic erosion in their center of diversity, and allow evolutionary processes that mold landrace diversity to continue, their value should be raised to approximate or exceed the social value of high-yielding wheat varieties. Understanding the different patterns of neutral and adaptive diversity, from the population- to the landrace-level, is essential to explain how landraces conserved on-farm will continue to evolve and how to minimize genetic erosion of this indispensable genetic resource. New strategies are emerging to produce modern landraces based on multiple crosses and selection from populations of einkorn, emmer, durum, and bread wheat in combination with on-farm site-specific selection to obtain highly adaptable genotypes for local and regional production. Participatory plant breeding and variety selection practices have emerged as a powerful strategy to merge breeders' knowledge and farmers' selection criteria, emphasizing decentralized selection in the target environments with the active participation of local farmers. Wheat breeders, seed producers, farmers and end-users, as stakeholders in participatory breeding, are involved in all aspects of research and development of new cultivars. Participatory plant breeding and variety selection are more successful than the classical approach used in high-input breeding programs for improvement in stress-prone environments where sustainability is a high priority. Despite being more complex to carry out, participatory plant breeding not only delivers improved germplasm, but also opens venues of communication and collaboration between farmers and other stakeholders for the benefit of all. Nonetheless, the main challenges of on-farm breeding and conservation of wheat landraces are non-biological, but involve a complex of ethno-anthropological processes, including legal, economic and social factors, superimposed on ecological and genetic processes. Wheat landraces having multiple home uses are more likely to be

conserved and sustainably utilized for the foreseeable future.

Acknowledgments

Thanks to two anonymous reviewers and to the editor of Emirates Journal of Food and Agriculture for their constructive editorial and review comments which contributed to the improvement of this article.

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Emir. J. Food Agric. 2013. 25 (1): 30-38
doi: 10.9755/ejfa.v25i1.5341
http://www.ejfa.info/

PLANT SCIENCE

Improper agricultural practices lead to landslide and mass movement disasters: A case study based on upper Madi watershed, Nepal

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Abstract

Damage, destruction, and fatalities related to landslide and mass movement are common phenomena in the Himalayan countries like Nepal, especially during the monsoon period. In Nepal, landslides represent a major constraint on livelihood and development, causing high levels of economic loss and substantial casualties each year. This fact was tragically illustrated on 3 August 2010, when Nang-Nung of Taprang, situated at the 22 km away from the Pokhara valley, upper Madi watershed, experienced a catastrophe landslide powered by the Madi river. The landslide and the debris flow caused widespread damage to life and property and destroyed valuable infrastructure in the area and the adjoining villages. 72 houses were swept away, 59 houses were partially damaged, 58.4 ha of land was washed away, 6 people were wounded, 5 people were killed, and many social infrastructures were damaged. The disaster was a result of a landslide triggered by heavy precipitation and stream undercutting the existing landslide.

Key words: Adaptive capacity, Debris flow, Landslide, Summer monsoon, Upper Madi watershed

Introduction

Nepal is a small landlocked country with a total area of 147,181 km^2 and shares its political borders with China in the north and India in the east, west, and south. The geographical structure of Nepal resembles a rectangle with three main ecological zones running horizontally as continuous belts, namely, mountainous, hills, and terai (Degen et al., 2010; Gautam et al., 2009). Table 1 provides a summary of geographical and demographical features of Nepal (CBS, 2003; FAO, 2002). Nepal is one of the least developed countries in the world with low Gross National Income (GNI) per capita of about USD 440 per person per year (WB, 2010). Although certain level of urbanization is desirable for the overall development of a country, Nepal remains one of the least urbanized countries in the world, where still more than 80% of the population live in isolated and remote rural areas (Tiwari, 2008; WB, 2010). Nepal has a very low Human Development Index (HDI) of 0.509, which is measured based on the average progress of a country in overall human development (UNDP, 2009). In addition, it was estimated that still more than 30% of the Nepal's population lives below the poverty line as of USD 1.25 a day (UNDP, 2009).

Nepal is located in the heart of the Himalayas. About 83% of the country is mountainous terrain and the remaining 17% is flat land i.e. alluvial plains (Ray and De Smedt, 2009). Geographically, Nepal is characterized by a transitional mountain area between the fertile Gangetic Plain of India and the arid plateau of Tibet, China which further lies in the tectonically active zone (Pokharel et al., 2009). The elevation of the country varies from 100 m in Terai to 8,850 m in the Himalayas (CBS, 2003). Due to the sharp vertical variation, Nepal is susceptible to various types of natural disasters, especially water-induced disasters such as landslide, slope failure, soil erosion, landmass movement, and flood. The watershed areas in the hills and mountains of Nepal are with most fragile ecosystems and poor agricultural potential due to weak geological structure, steep and rugged surface, and poor quality of soil (Pandit et al., 2007).

Received 15 November 2011; Revised 15 January 2012; Accepted 13 February 2012; Published Online 24 November 2012

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Table 1. Geographical features of Nepal.

Parameters	Mountains	Hills	Terai
Altitude (m above sea level)	4877-8850	610-4877	100-610
Area (km^2)	51,817	61,345	34,019
% of Total area	35	42	23
Width (North-south)	25-60	60-80	25-40
Climate type	Tundra	Temperate	Subtropical
Population (% of total)	7	44	49
Cultivated area (% of total)	0.3	48.1	51.6
Dominant agricultural system	Livestock-based	Cereals, horticulture, livestock	Cereals, cash crops, livestock

As depicted in Table 1, approximately 50% of the Nepal's total population lives in hills and mountainous regions, usually with small land holding and over 60% of dwellers in these regions are considered below the poverty line (DFID, 2005). Predominantly, agriculture is the main source of subsistence, often supplemented by animal husbandry for poor dwellers in the mountainous and hilly regions (Degen et al., 2010). While the agricultural production is mostly in subsistence level; the modern developmental sector is incapable to provide enough economic opportunities within the country for the huge mass of the ever increasing economically active population (Subedi, 2009) making the people living in hills and mountainous regions with high exposer and low adaptive capacity more vulnerable and susceptible to different types of natural hazards in the country.

Throughout the world, landslides are a major hazard in mountainous regions (Fort et al., 2010). Rugged topography, unstable geological structures, soft and fragile rocks, common earthquakes, active tectonics, and highly seasonal and intensive rainfalls during monsoon periods has made the fragile Himalayan environment vulnerable to hazards and disasters, especially landslides (Adhikari and Koshimizu, 2005; Chalise and Khanal, 2000; Dahal and Hasegawa, 2008). Landslides, monsoon floods, glacier lake outburst floods (GLOF) and debris flow disasters are common phenomenon in Nepal, since it is situated entirely in the Himalaya (Adhikari and Koshimizu, 2005). Nepal suffers from tremendous landslides and debris flow disasters every year with heavy loss in term of economic and physical structures. Great number of people are affected by large and small-scale landslides throughout the country as well (Adhikari and Koshimizu, 2005; Pokharel et al., 2009). Intense pressure on natural resources, mass poverty and subsistence agriculture, and recent development of infrastructures such as road networks and irrigation canals in unstable mountain terrain without proper engineering and safety measures have further aggravated the problems of surface runoff, erosion, landslides, sedimentation, and floods, especially in mountainous watersheds (Chalise and Khanal, 2000).

Landslides, floods, and mass movement disasters are common phenomena in the upper Madi watershed associated with extreme weather events, especially during the summer monsoon period. Every year, people from Sikles, Parche, Khilang, Tangting, Taprang, Sondha, Seti Khola, and Chansu areas experience unprecedented channelized debris flow, landslides, and floods, destroying life and property. However, the cumulative data on landslides, floods, and debris flow disasters and their effect on public health and environment of the upper Madi watershed is limited. In addition, there has been very little systematic documentation of these events and the previous natural history is not known. Thus, this paper presents an analysis of the landslide and debris flow disasters in the upper Madi watershed and the damage caused by them to the local livelihoods. The main objective of this study is to investigate impacts of landslide and mass movement disasters on the local livelihoods. The result may help to draw attention of government as well as volunteer organization in the affected area which is essential for future likelihoods of the area.

Materials and Methods

Study areas

The Madi watershed is located on the Gandaki Zone, Western Development Region of Nepal. It has a total area of about 1,123 km^2 with estimated population of about 0.2 million (Khanal and Watanabe, 2004; Poudel, 2000). The elevation of the watershed varies from 307 m in the south to 7,937 m in the north encompassing three major physiographical regions- the middle mountain, the high mountain, and the high Himalaya (Khanal and Watanabe, 2004). Geologically, the watershed is divided into three forms- the Lesser Himalayan meta-sediments in the south, the Higher Himalayan

crystalline, and Tibetan sedimentary Tethys sediment in the north (Khanal and Watanabe, 2004). The average annual precipitation in the watershed is about 4,000 mm in which more than 75% of the total annual precipitation occurs during the four months in summer (June–September) (Poudel, 1996). The topography of the watershed is very rugged and the Lesser Himalayan consists of intensively folded meta-sedimentary rocks whereas the Higher Himalayan crystalline consists of Precambrian high grade metamorphic rocks (Khanal and Watanabe, 2004; Poudel, 1996). Similarly, the Main Central Thrust (MCT) crosses in the east-west direction in the central part of the watershed (Khanal and Watanabe, 2004).

Madi River, one of the major tributaries of the Narayani River of the Gandaki basin after mixing with the Seti River, with average drainage density of 2,467.07 m/km^2 flows through the Madi watershed (Poudel, 2000). The area is inhabitated by more than 16 ethnic groups and predominantly, agriculture is the main source of subsistence, often supplemented by animal husbandry and natural resources. On the basis of elevation, the agricultural pattern has been classified into lowland (at 1,000 m or below) and highland agricultural land (above 1,000 m). Highland agricultural areas are generally called *bari* (non-irrigated land; mainly harvest maize, millet, barley, wheat and buckwheat) whereas lowland is called as *khet* (irrigated land; mainly harvest rice) (Figure 1).

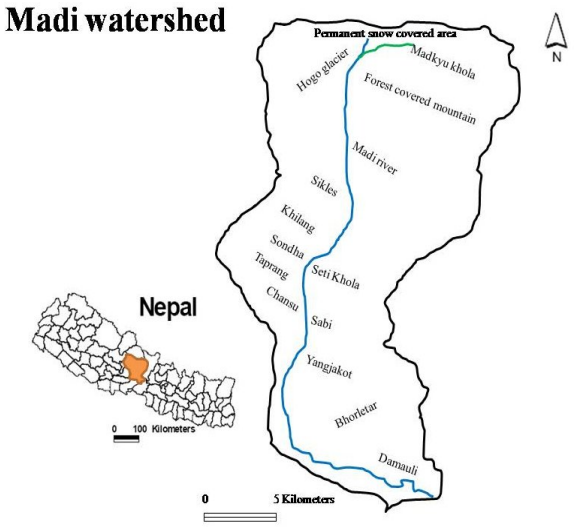

Figure 1. Location of study area, upper Madi watershed.

Data collection

Information and data quoted in this paper was collected during a baseline survey of 300, randomly selected households in Sikles, Tangting, and Taprang. A mixture of data collection methods were employed during the fieldwork from August 20–November 20, 2010. These included an in-depth household interview, a key informant opinion, and a focus group discussion (FGD). The survey was an integral part of the evaluation and was aimed at identifying the impacts of natural disasters in the studied areas. While this study incorporates both quantitative and qualitative methods more weight was put on the qualitative data.

Prior to primary data collection, one of the authors interacted with youth group, women group (especially mothers' party), community leaders, and village development committees (VDCs) officials in order to get acquainted and to explain goals of the research.

In order to evaluate the impact of landslides and debris flow disasters in the upper Madi watershed, an in-depth household interview was carried out by employing a self-administered questionnaire survey. A total of 300 households were randomly selected for the face-to-face questionnaire interview in three villages. The questionnaire was designed to assess impacts of natural disasters in the vulnerable areas of our target population and consists of different parameters. The first part of the household questionnaire concerned demographic structure such as caste, gender, family member, and occupation. The second part of the questionnaire concerned agriculture practices such as type of land ownership, cropping pattern, production of food grains, and numbers of livestock. The third part of the questionnaire concerned socio-economic information such as health, education, annual income, and sources of income. The fourth part of the questionnaire concerned issues related to natural disasters such as causes of landslides and debris flow, impacts of these disasters on the local livelihoods, adaptive capacity to cope with the disasters, and involvement of government in tackling the problems.

Similarly, elicited detailed qualitative data about rural livelihood experiences was also collected through informal, loosely structured, and open-ended interviews. A total of 20 key informants were selected from a range of categories in order to represent the broad interests and perspectives in studied communities. The interviewed key informants were: local entrepreneurs, school-teachers, youth leaders, mother's leaders, and elderly community members. Additionally, FGD was carried among villagers to

encourage the participation of occupational castes and to hear their voices regarding their access to local resources and their involvement in decision making system.

Results and Discussion

On 3 August 2010 the "Nang Nung" of Taprang which is commonly called as "Taprang ko Pahiro" (Pahiro means landslide in Nepali) experienced an unprecedented landslide and channelized debris flow powered by the Madi river. According to the local dwellers, there was sustained rain over the preceding few days and heavy rainfall and higher stream discharge was observed on the evening of 2 August 2010. There was thunder and lightning and the area experienced threatening noise and ground shaking approximately on the early morning (5:00 am) of the day of the incident day. The landslide or mass movement occurred shortly after the cessation of the sounds and shaking; at 8:45 am of 3 August 2010. The sudden surge of debris flow inundated the part of the Madi river and carried much of it into the Madi river thereby blocking the river. Field observations confirmed that this landslide was shallow and mostly translational in nature, and expanded 1,600 m in length and 600 m in width, respectively (Figure 2). On the other hand, the Madi river was blocked by the debris (logs and sediments) for several days (20 days) particularly at the narrow sections of the channel resulting drastic change in the morphology of both the channel and flood plains even far downstream areas during this period (Figure 3).

Figure 2. Nang Nung Taprang landslide.

The water volume in the Madi river increased tremendously resulting a lake with width 80 m, length 1,500 m, and depth of 5 m, respectively. Additionally, due to blockage of the river, the water had collected nearly in 3 km of the river section. The landslide claimed life of five people, two of them were school children from Taprang and three of them were from Sikles heading towards Pokhara. The landslide caused high levels of economic loss and substantial numbers of fatalities in the area: 6 people were wounded, 72 households were displaced (61 from Taprang and 11 from Seti Khola), 59 households were partially damaged, 58.4 ha of agricultural land were swept away, 1.5 km of Kahu Khola - Dudhpokhari Rural roadway had been swept away, a suspension bridge was swept away that used to connect Namarjung VDC with Sildujare VDC, and many social infrastructures like school, community buildings, post office, and other physical structures were damaged (Figure 4).

Figure 3. Blockage of Madi river by the debris flow in Nang Nung Taprang.

Figure 4. Houses washed away by torrential flood in Sondha.

Approximately 1.5 km walking trail (from Taprang to Sikles) was washed away by the floods. 200 houses were evacuated in the affected area due to blockage of the Madi river (Figure 5). In addition, the blockage of the Madi river affected the downstream VDCs such as Ghumle Bazar, Chanchu, Bhainse, Majhthana, Hansapur, Thumki, and Mijure (Figure 6).

Figure 5. Disruption of gravel road due to landslide in Taprang.

Figure 6. Temporary bridge in the Madi river after the suspension bridge has been washed away.

In the Madi watershed, gently sloping land (<5°) comprises relatively low (9%) portion of the total basin area followed by moderately to steeply sloping (5-30°) mountain terrain and very steeply sloping (>30°) mountain terrain with rock headwalls (Khanal and Watanabe, 2004). “Slope Instability” is one of the important parameter which gives rise to gravity-dominated down-slope movements of ground-forming materials without the primary assistance of a fluid-transporting media (Poudel, 1996). Generally, all slopes are under stress owing to the force of gravity and once the forces acting on a slope exceed the strength of the materials forming the slope, then the slope will fail and thus, movement occurs (Jones, 1993; Poudel, 1996). Approximately, more than half of the total area falls under the inclination of 60% and above and thus, the length of slope and vertical exaggeration of steepness favors more susceptibility of hill-slope instability in the Madi watershed basin (Poudel, 1996).

Geologically, the upper Madi watershed is narrow, steep, and bounded by high rugged mountains. The Higher mountain slopes of the Madi watershed are composed either of bare bedrock or more commonly of bedrock mantled by thick to very thick unconsolidated soil cover. In addition, due to steep gradients, the rivers are energetic, producing high runoff and sediment discharge, especially during monsoon period. A combination of geologically active with glacially oversteepened slopes and deep gorges in places, rugged topography, and highly seasonal and intensive rainfall has made the region more vulnerable to hazards and natural disasters. Extreme weather events associated with the seasonal heavy rainfall is the principal causes of landslide and mass movement in the Madi watershed. Up to some extent, physical weathering of rocks in the Madi watershed basin is also responsible for contributing landslide and debris flow disasters in the area. In addition, deforestation, terracing, and the improper land use practices make the upper Madi watershed area most unstable landscapes that often lead to slope-failure. Due to marginalization of agricultural system, farmers were compelled to construct new terraces towards the toe of the old terraces which are highly susceptible to failure and also, most of these terraces slump down during the summer heavy monsoon rainfall. Since the upper Madi watershed region is rugged, steep, and narrow, a relatively small volume of material is sufficient to dam the Madi river. On the other hand, the lower Madi watershed slopes are sparsely vegetated along with dense settlement on the bank of the Madi river, and hence the incident of landslide and debris flow disasters often toll either of human lives or physical properties on the downstream as well.

Anecdotal evidence suggested that the upper Madi watershed has been suffering from tremendous landslides and debris flow since 1956. Based on the aerial photographs, a total of 236

active landslides and debris flow scars were identified in 1956/58, 18 in 1972, 5 in 1979, and 25 in 1996 in the Madi watershed (Khanal and Watanabe, 2004). Most of these landslide and debris flow scars were concentrated mainly in Sikles, Saimarang, Bhujung, Karapu, and Jita area (Khanal and Watanabe, 2004). Both natural and anthropogenic activities were responsible for these natural hazards and disasters in the past decades. Since majority of the people derive their livelihood from agriculture, paddy cultivation is one of the important farming practice in the locality. However, the inappropriate cropping system, especially paddy cultivation may favor the activity of slope-failure. Since water has to be retained for paddy cultivation in which the retaining water percolate down the rock strata and assist the weathering and paddy cultivation terraces are distributed even above 60% hill-slope gradient in the Madi watershed (Poudel, 1996). A study carried out by Poudel (2000), found that the Madi watershed has the maximum share of its area under the very high potential hazard zone. According to nature of terrain properties, Poudel (2000) divided the Madi watershed into five hazard class in which only 0.25 % of the area has very low hazard potential followed by low (22.66%), medium (35.74%), high (32.26%), and very high (9.09%), respectively. Thus, majority of the area are under the fragile environment vulnerable to hazards and natural disasters at the Madi watershed.

Altogether there are 25 settlements in very high hazard-prone zone in the Madi watershed (Poudel, 2000). The most vulnerable sites are Taprang of Sildujare VDC, lower part of Khilang, Sondha, Seti Khola, Chansu, Sabi, Pakhagaun of Saimarang VDC, Ghartidanda of Bhachock VDC, and Harse of Mijuredanda VDC. However, in recent years, three VDCs namely Parche, Namarjung, and Sildujare were severely affected by the landslide and debris flow disasters in the area. According to our fieldwork, the most vulnerable sites were Sondha, Taprang, Seti Khola, Chansu, and Sabi in the upper Madi watershed. It was observed that many existing landslides were started from the paddy terraces in the Madi watershed. The big “Nang Nung” slide in Taprang, landslide in upper Sondha, and landslide in lower Chansu were the prominent examples of slope failure in the upper Madi watershed which originated from the paddy field. According to the anecdotal evidence from the experience of local dwellers, Taprang village has been severely affected by the landslide and mass movement and there has been an increase in the number of such disasters over the past few decades. According to the interviewed key informants, the Nang Nung landslide in Taprang was initiated around 1973. Since then, the volume of the landslide has been increasing every year due to debris flow and stream side-cutting. From the experienced of local people, landslides occur frequently in this area during the monsoon season of July to September, usually leading to interruption of the transportation.

In 2003, another big incident occurred in the upper Madi watershed severely affecting Sondha, Chansu, and Sabi area. In the month of August 2003, after a torrential rainfall, the water volume in the Madi river increased tremendously and the river lost its regular course. The incident occurred during the night and the flash flood claimed life of 4 people in the Chansu area. Those who were asleep had no time to wake up, and those who were awake had no idea about what was happening in the darkness. The flood swept away a bridge over the Sondha, 20 livestock in the Sabi, and a major portion of Kahu Khola to Sondha road was washed away. Apart from the loss of lives and damage to infrastructure and property, the human suffering was tremendous because the flood washed away many houses and agricultural land in the affected areas. In the mountainous countries like Nepal, water-induced disasters such as landslides, debris flows, mass movement, and all types of floods are chronic hazards during the monsoon period causing widespread damage (Ray and De Smedt, 2009).

Generally, landslide and mass movement occur when the soil pores are saturated with water and sliding component of the mass becomes dominant than the vertical component (Pokharel et al., 2009; Poudel, 1996). The impacts of disasters are further aggravated by rapid and uncontrolled development as well as anthropogenic activities including deforestation, encroachment of the forest land for cultivation, and infrastructures development in weak and unstable terrain (Pokharel et al., 2009; Ray and De Smedt, 2009). Therefore, the combined effect of steep slope and heavy precipitation events enhance soil erosion and bank cutting which ultimately leads to mass movement or landslide.

Like in the upper Madi watershed, initiation of many small and shallow landslide and mass movements during and after highly localized torrential precipitation events were also reported from other areas of Nepal (Adhikari and Koshimizu, 2005; Pokharel et al., 2009). On 22 July 1996, Larcha experienced an unprecedented channelized debris flow down the valley of the Bhairab Kunda stream after heavy rainfall and 54 people were killed in the incident (Adhikari and Koshimizu, 2005). In another incident, the devastating flood and the

landslide due to heavy rainfall for two consecutive days in the central region of Nepal (Bagmati, Kulekhani, and Narayani watersheds) in 1993 killed 1,160 people and affected 70,000 people (Pokharel et al., 2009). Similarly, heavy rains from the tail end of the monsoon in October 2009 caused floods and landslides in the mid- and far-western regions of Nepal, thereby killing a total of 78 people, injured 37 people, affected 175,000 people (29,141 families), and displaced 15,000 people (2,802 families) (IFRC, 2010). Due to extreme weather events such as landslides, floods, and avalanches, Nepal had to bear estimated loss of 21.2 million rupees in 1991 and 4904 million rupees in 1993 with average annual loss of 752.2 million rupees between the years 1983 and 1998 (Chalise and Khanal, 2000). Similarly, approximately 230 persons were killed by these disasters every year in Nepal between the years 1983 and 1998 (Khanal, 1999).

Generally, the victims of extreme weather events were those communities who heavily relied on natural resources, especially subsistence farmers and those living on unsustainable land, often unstable and flood prone areas lacking infrastructures (Chalise and Khanal, 2000; POSTNOTE, 2006). The poorest inhabitants of developing countries, especially those in the least developed countries (LDCs) were vulnerable to natural disasters before and after the events due to the low adaptive capacity to cope with the disasters (POSTNOTE, 2006). Although the frequency of occurrence of natural disasters is very high, the relief provided by the Nepal government to the affected area has been very small and uneven. In addition, geographically, the affected areas are very much scattered, isolated, and remote and often without road network that substantially prevent aid and other helps reaching the affected areas (IFRC, 2010). The upper Madi watershed had always received less attention from government and other voluntary organization during the past events. However, in the recent Taprang landslide of 3 August 2010, the affected areas were helped by individuals and different organizations (Table 2).

Table 2 clearly illustrated that non-governmental organizations are more reliable and active in providing financial aid and relief to the areas affected by natural disasters in the mountainous watersheds. Although the downstream impacts of upstream interventions are high in the discourse of natural disasters, there are very few programmes and achievements in terms of linking the two. The trickle down effects of water conservation, reduced soil erosion, and well managed agricultural practices will hence decrease sedimentation in the plains and reduced potential of hazards in the downstream when upstream watersheds are managed properly (Pandit et al., 2007). However, there is no such coordination among settlers in the upper Madi watershed and the magnitude of natural hazards and disasters is always unpredictable and widespread. Since there is always experiencing the losses of life and property as well as the damage to scarce agricultural land and costly infrastructures due to extreme weather events, especially landslide and flood, a large scale of outmigration has been taken place from those areas which were severely affected by landslide and flood. A large volume of households have migrated to Pokhara from Taprang and Sondha areas since the settlements in the areas have been threatened due to reactivation and enlargement of existing landslides.

Table 2. Funds collected for the victims of Taprang landslide, 2010.

Donor	Amount in rupees	Remarks
Pahar Trust Nepal: UK based International Non Governmental Office	612,576	To build new houses in Taprang and Seti Khola area for affected households
Nepal Red Cross Society	300,000	Will be used for the affected areas
John Riley Isle man of UK	100,000	Will be used for the affected areas
Taprang Pokhara Samparka Samaj	200,000	Will be used for the affected areas
District Development Committee	200,000	Will be used for the affected areas
Central District Officer (CDO) office of Kaski	25,000	Each families were given 25,000 who lost their lives in the landslide
Village Development Committee	Unknown	Provided to the families who lost their lives in the landslide
Nepal Red Cross Society	Unknown	Provided to the families who lost their lives in the landslide
Sikles village raised a fund	300,000-20,000	Three families from Sikles were given to 100,000/person died in the landslide and 10,000/person died in the landslide from Taprang

Nevertheless, outmigration is not a solution for mitigation to the affected areas. Although local dwellers are aware about the frequent problems of landslide and flood, still disasters control and mitigation as well as adaptation and management activities in organized way have not yet received priority in the upper Madi watershed. According to the interviewed key informants, there are no facilities to record hydro-meteorological data which can introduce an early warning system to reduce the impact of natural disasters. Due to lack of both technical as well as financial capability to cope with the natural disasters, the vulnerability of life and properties in the upper Madi watershed has been increasing continuously. The general consensus is that an improved knowledge about the frequency, magnitude, causes, and consequences of natural hazards and disasters is essential to develop short- and long-term mitigation measures (Chalise and Khanal, 2000). Thus long-term monitoring of climatic, hydrological, and other critical parameters in selected river basins is absolutely essential to reduce the risks and impacts of these natural disasters. The underlying causes and processes of landslides, mass movements, and floods need to be well studied to develop mitigation strategies and reduce the magnitude and impact of these disasters. It is also essential to cooperate with communities in order to control landslide, mass movement, and flood to minimize the loss of life and properties. Moreover, these community based institutions should be strengthened with provision of training and other necessary support including financial and manpower during the disasters period (Khanal and Watanabe, 2004).

Conclusion

Landslides, mass movements, and floods associated with extreme weather events are common phenomena in the mountainous terrain of Nepal. Torrential rainfall is the principal triggering factor for landslides, mass movements, and floods that often cause high levels of economic loss and substantial numbers of fatalities as well as destroy infrastructures and properties. Our study revealed that the upper Madi watershed has a greater number of landslides with extensive coverage. Each year, the occurrence of natural disasters, especially landslide and flood threatened the settlements by damaging agricultural land, properties and human life. Although the upper Madi watershed has been experiencing landslide and flood for more than half century, there are still no concrete strategies to mitigate the problems. There are no facilities to record and monitor the climatic, hydrological, and other critical parameters to introduce an early warning system. Moreover, the area received less priority in terms of relief and aid from Nepal government before and after the extreme weather events. Because of such discouraging government interventions as well as reactivation and enlargement of existing landslides, the vulnerability of life and properties have been increasing in the upper Madi watershed.

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doi: 10.9755/ejfa.v25i1.15395
http://ejfa.info/

PLANT SCIENCE

Potassium dynamics in three alluvial soils differing in clay contents

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Abstract

Despite the presence of a huge amount of potassium (K^+) in the soil, most of the soils are deficient in plant available K^+. A large amount of the K^+ is fixed by clay minerals present in such soils and cannot be taken up by plants to achieve optimum plant growth. In such type of soils, large amount of K^+ fertilizers are required for optimum plant growth, as plants do not respond enough to a normally recommended K^+ fertilization. Vermiculite clay minerals can fix an enormous amount of applied K^+, which becomes slowly available to the plants. The K^+ dynamics in such soils are valuable to recommend K^+ fertilizer requirements for sustainable nutrient management. We analyzed the K^+ dynamics of three alluvial soils, i.e Kleinlinden, Giessen and Trebur, collected from Germany and found that the soils with vermiculite and smectite clay minerals have more K^+-fixing ability than soils dominated by illite clay minerals. However, as the K^+ concentration decreased in the soil solution, smectite-dominant soils may easily release fixed K^+ due to lower particle-charge, whereas vermiculite and illite dominant soils may not release fixed K^+ easily. Moreover, ammonium exchangeable K^+, non-exchangeable K^+, total K^+ and K^+-fixing capacity of these soils are directly proportional to the soil clay contents. While recommending K^+ fertilizers clay contents and the type of clay minerals is not considered and recommended K^+ fertilizers sometimes do not response plant growth enhancement. Therefore potassium fertilizer should be recommended by taking into consideration the type and amount of clay minerals present in the soil.

Key words: Alluvial soils, Clay, Electro-ultra-filtration, K^+-dynamics, K^+-fixing soils

Introduction

Potassium (K^+) is the most abundant macro plant-nutrient in most soils. It is crucial since it serves three important functions i.e. enzyme activation, charge balance and osmotic regulation in higher plants (Mengel, 2007). Its concentration in the earth's crust is 2.3%, but the greatest part of this K^+ is bound to primary and secondary clay minerals, and thus not readily available for plants. Its availability to plants depends upon the K^+ concentration in the soil solution and transfer of K^+ from exchangeable and fixed form to soil solution. The concentration of K^+ in soil solution is referred to as "intensity", whereas the soils "capacity" is the total amount of K^+ in the soil which can be taken up by plants. The transfer rate from "capacity" to "intensity" reflects the kinetic factor of renewal of potassium (Barber, 1984).

The major natural source of soil potassium is the weathering of K^+-containing minerals such as micas and alkali feldspars, which contain 6 - 9 and 3.5 - 12% K^+, respectively. During K^+ uptake, plants reduce its concentration in the immediate vicinity of roots which releases K^+-ions from the minerals (Kuchenbuch and Jungk, 1984). The release of K^+ converts micas to secondary 2:1 clay minerals illite and then vermiculite (Figure 1) (Havlin et al., 1999). Application of K^+ fertilizer to soils containing illite and vermiculite clay minerals often leads to fixation of some of its fraction by soil particles. This fraction then becomes unavailable or slowly available to the plants (Scott and Smith, 1987). The fixed K^+ can be made available to plants by its release from soil particles into soil solution when the concentration of K^+ is lowered in soil solution (Cox et al., 1999), but in many cases this release is too slow to meet the plants requirement.

Potassium sorption on exchange sites and its fixation depend on the physicochemical properties of the soil, as well as type and content of the clay minerals (von Braunschweig, 1980).

Received 16 April 2012; Revised 29 June 2012; Accepted 15 July 2012; Published Online 24 November 2012

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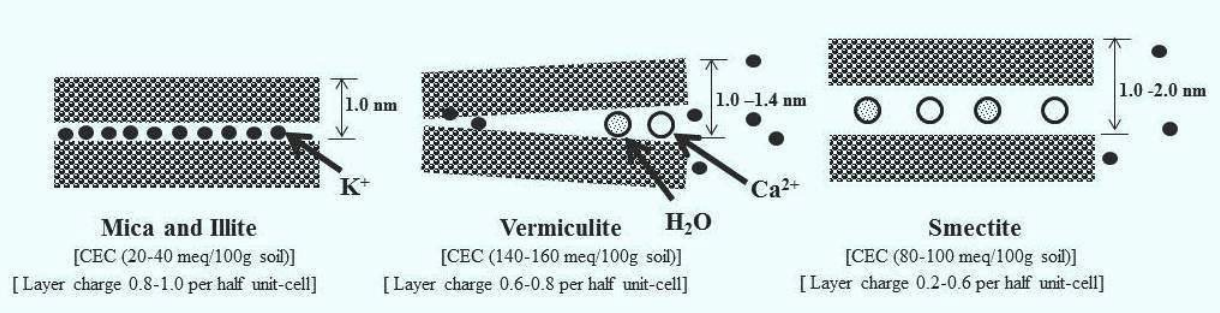

Figure 1. Properties of different types of clay minerals developed by weathering of Mica.
(modified after Wakeel et al., 2011).

The soils containing vermiculite clay minerals are able to fix huge amounts of K^+. Their cation exchange capacity (CEC) is 1.2 – 1.5 mol (+) kg^{-1} soil and the layer basel distance for such soils is 1.4 – 1.5 nm with a layer charge of 0.6 – 0.9 per half unit-cell. Other K^+-fixing clay minerals 'smectites' have CEC 0.8 – 1.2 mol (+) kg^{-1} soil, layer thickness 1.0 – 2.0 nm and layer charge 0.2 – 0.6 per half unit-cell (Bohn et al., 2001).

Sometimes plants do not respond to the application of generally recommended levels of K^+ fertilizer in the soils with expandable clay minerals (Mengel and Kirkby, 2001). Doll and Lucas (1973) reported from Michigan that in a sandy clay loam soil about 92% of the applied K^+ fertilizers were fixed and 1600 kg K^+ ha^{-1} were applied to make it responsive for tomato production. This soil was rich in illite and vermiculite clay minerals with high CEC and a major part of applied K^+ was fixed immediately and became unavailable to the plants. Similar results have been obtained in other parts of the USA (Mengel and Kirkby, 2001).

The fate of K^+ fertilizers applied to the soil depend upon the clay contents, clay minerals and fractions of K^+ already available in the soil. This study shows the K^+ fractions and its correlation with the clay contents of three different soils of Germany differing in clay contents. The aim of the proposed study was to extract the information for precise K^+ fertilizer recommendations may be helpful for research experiments to be conducted for optimal K^+ management in the soils differing in clay minerals for better plant growth and yield production for farmer community as well (Moyin-Jesu, 2008).

Materials and methods

Physicochemical properties of the soil

Three soils i.e. Kleinlinden, Giessen and Trebur, differing in clay contents, were selected for the study. The soils are named based on the location name. Kleinlinden is a subsoil while Giessen and Trebure are surface soils (up to 45 cm depth). The pysicochemical properties of these soils are enumerated in Table 1.

Analysis for inorganic cation (K^+, Na^+, Ca^{2+}, Mg^{2+})

Finely ground 10 g soil and 50 mL 1 M NH_4-acetate were shaken for 1 h on a mechanical shaker. After filtration from white-band 589 filter paper (Schleicher and Schuell Co., Dassel, Germany) cation concentrations of K^+, Na^+, Ca^{2+} and Mg^{2+} were determined by means of atomic absorption spectrophotometry (SpectrAA 220FS, Varian) (Igwe et al., 2005).

Determination of K^+-fixing capacity of soil

Fine ground 10 g soil was shaken for 1 h on a mechanical shaker with 50 mL 0.005 M KCl in Erlenmyer flask. The sample was oven-dried at 100°C and 50 mL 1 M NH_4-acetate solution were added followed by 1 h shaking on a mechanical shaker. After filtration through white-band 589 filter paper (Schleicher and Schuell Co., Dassel, Germany) the samples were analyzed for K^+ concentration using atomic absorption spectrophotometer (SpectrAA 220FS, Varian).

Table 1. Physicochemical properties of soils i.e. Giessen, Kleinlinden and Trebur.
Clay-mineral types are according to Paul, (1989).

Soils	pH	Clay	Silt	Sand	Soil textural class	Dominant clay minerals
		($g\ kg^{-1}$)				
Kleinlinden	5.8	207	338	455	loam	Illite
Giessen	5.2	303	631	47	silty clay loam	Vermiculite & smectite
Trebur	7.4	446	436	112	silty clay	Smectite

K^+ fixing capacity was calculated by using the formula (Wakeel, 2008);

K_{fix} (µg /g or mg kg^{-1}) = (9800 + K_a - K_r)/10

Where,

9800 = µg of K^+ in 50 mL of 0.005 M KCl solution

K_a = Exchangeable K^+

K_r = K^+ concentration in soil filtrate after fixation on soil particles

Note: Three replications for each soil were processed and mean values were compared.

Total cation concentration in the soil

Samples of finely ground soil (100 mg) were placed in a 30 mL platinum crucible. After addition of few drops of water, 5 mL of 48% hydrofluoric acid (HF) and 0.5 mL of 72% perchloric acid ($HClO_4$) were added. Soil acid mixture was heated on a hot plate until fumes of $HClO_4$ appeared. Crucibles were cooled, and then 5 mL of HF was added. The crucibles were placed in a sand bath covered about 9/10 of the crucible top with a Pt lid. Crucibles were heated to 200 to 225°C and the contents were evaporated to dryness. After cooling the crucibles, 2 mL of water were added followed by few drops of $HCLO_4$. Crucibles were placed again in the sand bath and the contents were evaporated to dryness. The crucibles were removed and 5 mL 6 N hydrochloric acid and 5 mL of double deionized water were added after allowing it to cool. The crucibles were then heated over a burner until the solution started boiling gently. When the residues were completely dissolved in HCl, the material was filtered into a 100 mL volumetric flask and cation concentration (K^+, Na^+, Ca^{2+} and Mg^{2+}) was measured in the filtrate by Atomic Absorption Spectrophotometery (SpectrAA 220FS, Varian) (Jackson, 1958).

Determination of interlayer K^+

Interlayer potassium was determined by means of electro-ultra-filtration (EUF) technique from the soils used for the various experiments (Schubert et al., 1989). The principle of EUF technique is presented in Figure 2. The soil suspension was exposed to an electric field and K^+ ions migrated to the cathode, where they were collected. Soil samples were extracted for 60 min at 20˚C and 200 V followed by extraction for 60 min at 80˚C and 400 V. Extraction was carried out at 10 min intervals so that 12 sub samples were obtained. Collected extracts were filled up to mark in 200 mL volumetric flasks and were analyzed for K^+ by Atomic Absorption Spectrophotometry (SpectrAA 220FS, Varian). The dynamics of available K^+ was considered for the six samples obtained in the first 60 min at 200 V and the dynamics of non-exchangeable K^+ release was obtained by considering the data obtained in following 60 min at 400 V. Extracted K^+ was plotted against time to characterize the desorption dynamics.

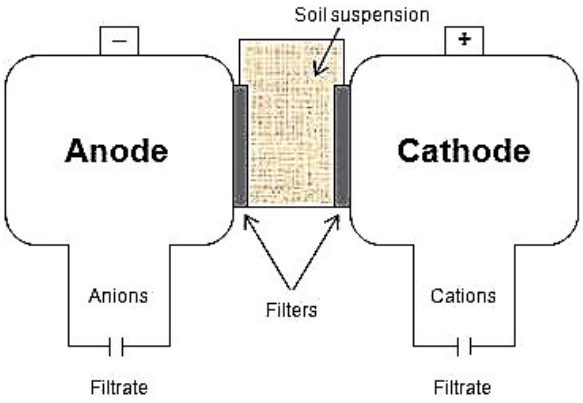

Figure 2. Scheme of the electrofiltration (EUF) apparatus. Migration of ions to electrodes results in dilution of ions in the solution and thus promotes the desorption of adsorbed ions
(Mengel and Uhlenbecker, 1993).

Results and Discussion

Comparison of K^+ release from the soils

The soils were characterized according to potassium (K^+) release from the clay minerals of the soils. Figure 3 shows that K^+ release was almost similar in soils Kleinlinden and Giessen (10 to 20 mg K^+ kg^{-1}). However, K^+ dynamics were different in the soil Trebur i.e. 35 to 165 mg K^+ kg^{-1} (Wakeel et al., 2005).

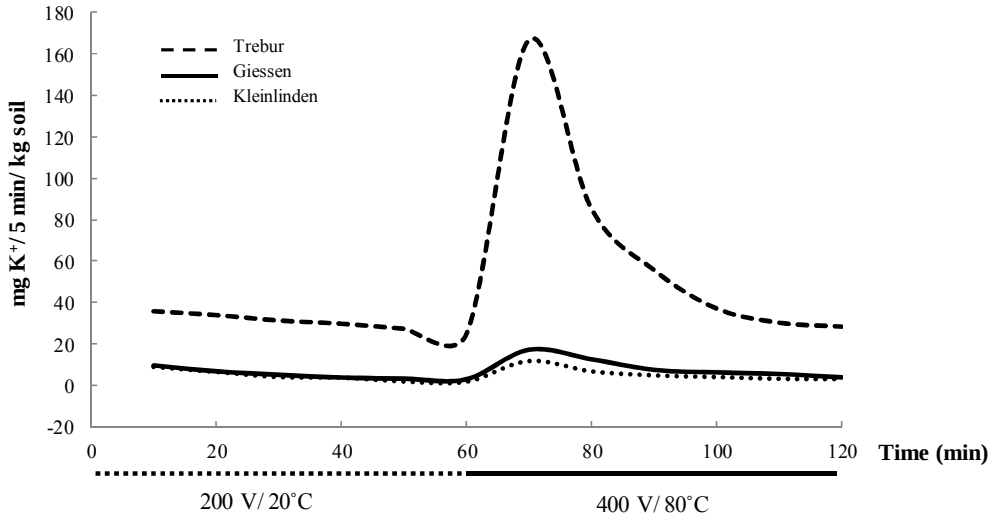

Figure 3. Potassium release from the soils (Kleinlinden, Giessen and Trebur) used for soil culture experiments. Potassium was extracted from the soils by electro-ultra-filtration (EUF) technique and K^+ concentration was measured with an atomic absorption spectrophotometer.

In the first 60 min, K^+ was extracted from all three soils at 200 V and this amount is known to be easily available for plants. If we compare this value obtained in soil Trebur with the other two soils, the easily available K^+ in soil Trebur was ~33 mg K^+ kg^{-1} soil, which was significantly higher ($p = 0.05$) than in soils Giessen and Kleinlinden (~10 mg K^+ kg^{-1}). Similarly, when the soils were exposed to 400 V from 60 - 120 min and K^+ release was measured from the filtrate. This was again almost similar for soil Giessen and Kleinlinden (~20 mg K^+ kg^{-1}), but the difference with soil Trebur was higher ($p = 0.05$), about 160 mg K^+ kg^{-1}, and afterwards this release was almost constant at the concentration of 40 mg K^+ kg^{-1} soil. Potassium extracted at 400 V is considered as slowly available K^+. Total amount of K^+ present in the soils, i.e. 14.5 - 16.5 g kg^{-1} do not show much variation between the soils, however the NH_4^+-exchangeable K^+ was much higher in the soil Trebur (Table 2). We can attribute higher K^+ values in first 60 min to higher exchangeable K^+, however higher values in the next 60 min show the characteristic of smectite clay minerals dominantly present in this soil (Paul, 1989). Such types of soils are able to release from total K^+ present in the soil when its concentration decreased in the soil (Schubert et al., 1989). Earlier in a field study no difference was found in plant growth and beet yield of sugar beet treated with 0, 400 and 1575 kg K^+ ha^{-1}, although the initial analysis of soil showed K^+ deficiency (Wakeel, 2008). We suggest that more interlayer space and the lower layer charge (Table 1) for smectite clay minerals was a factor for K^+ release on decreased K^+ concentration in the soil solution (Hinsiger and Jaillarr, 1993).

Clay contents and K^+ concentrations in the soils

The clay contents play an important role in K^+ availability to plants in the soils. Clay contents in the experimental soils were in the order, Trebur > Giessen > Kleinlinden. Total, fixed, and ammonium exchangeable K^+ concentration were directly proportional to the total clay contents of the soils. Figure 4A, 4B, and 4C show the correlation between clay contents and total potassium in the soil. The results revealed that more clay contents possessed more K^+ contents whether fixed or exchangeable (Jalali and Zarabi, 2006).

Table 2. Potassium dynamics in the soils and the K^+-fixing capacity of soil. The values are the mean of four replicates.
Means followed by the same letter in the same soil are not significantly different according to the LSD test at 5% level of probability.

Soils	$K^+_{(Total)}$	Fixed K^+	$K^+_{(Exch.)}$	K^+-fixing cap. of soil
	g kg^{-1}		mg kg^{-1}	
Kleinlinden	14.50 b	14.41 b	85 c	488 c
Giessen	14.90 b	14.80 b	98 b	526 b
Trebur	16.50 a	16.11 a	386 a	618 a

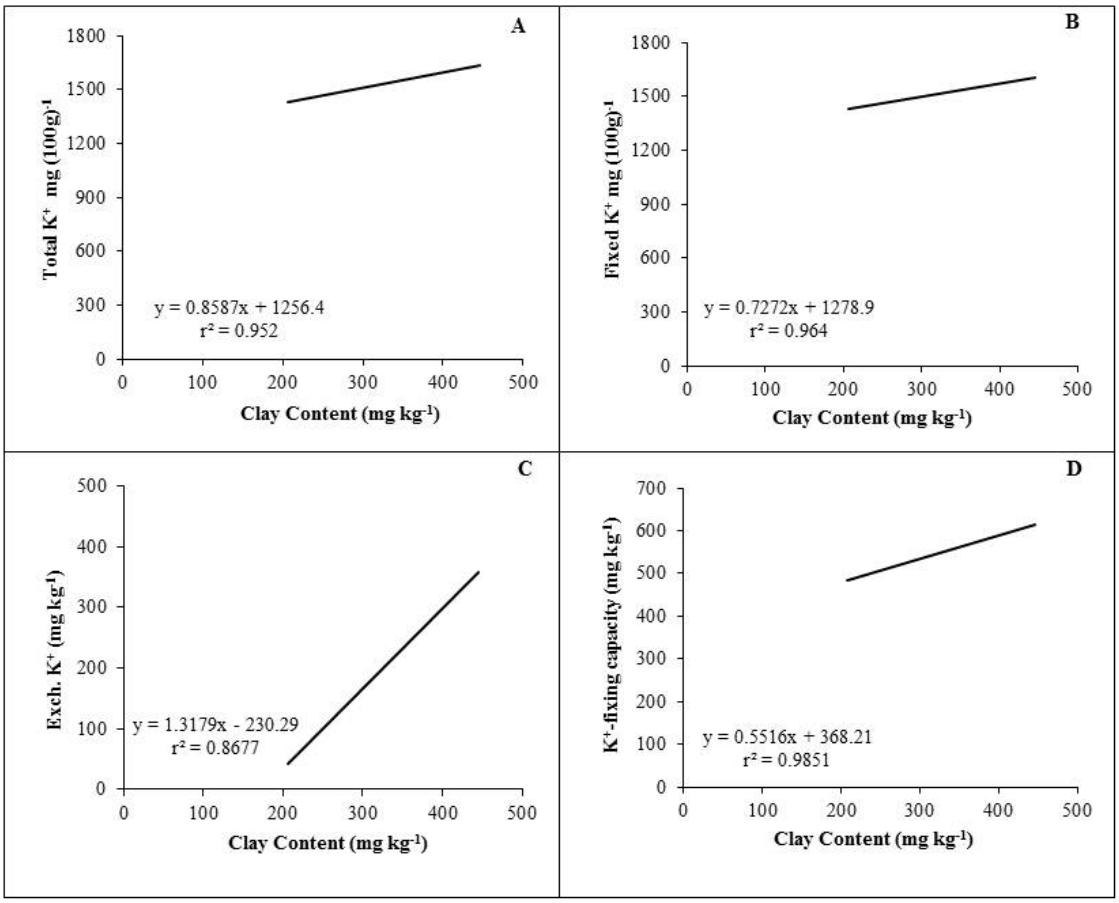

Figure 4. Correlation between soil clay contents and K^+ concentrations in the soils. A shows correlation between soil clay contents and ammonium exchangeable K^+, B shows correlation between soil clay contents and total K^+, C shows correlation between soil clay contents and fixed K^+ and D shows correlation between soil clay contents and K^+-fixing capacity of soil.

Possession of K^+ in different clay minerals was significantly different. The illites are contracted clay minerals with layer thickness of 1 nm. This is due to more interlayer K^+ ions than that in vermiculite and smectite. These all three clay minerals are developed from mica by its weathering. Mica is rich in interlayer potassium (Brady and Weil, 2001). Weathering occurs by release of interlayer K^+ and adsorption of other cations and water molecules.

Ammonium-exchangeable K^+ in all three soils is shown in the Table 2. Clay particles have -ve charge and have capacity to absorb K^+ ions. The soils with more clay contents (Trebur and Giessen) have more exchangeable K^+ as compared to soil with low clay contents (Blake et al., 1999). In Fig. 4C, a highly significant correlation (r^2= 0.87) between soil clay contents and exchangeable K^+ can be seen. In fact on the clay minerals, there are three types of K^+ sites. The first is planar (p) which is not specific for K^+ binding, second is edge (e) positions which are more selective to K^+ and third is inter lattice (i) with highest K^+ selectivity (Rao et al., 1999). Potassium ions present on position (p) and (e) are exchanged by NH_4^+ but K^+ present at position (i) are out of NH_4^+ reach. Higher concentration of exchangeable K^+ in Trebur soil may be due to the presence of smectite clay minerals. Low layer charge leads to less contraction and more interlayer spaces, which keep the K^+ ion reachable to be replaced by NH_4^+.

Relation between clay contents and K^+-fixing capacity of soil is also positively correlated (r^2=0.98). Clay contents have the ability to fix more K^+. Instead of positive correlation, the critical analysis of this data showed that K^+-fixing capacity was not only a function of clay contents. The clay contents in Trebur, Giessen and Kleinlinden soil were 207, 303 and 446 g kg^{-1}, respectively and K^+-fixing capacity was 488, 526 and 618 mg K^+ kg^{-1}

soil, which was 2.4, 1.7 and 1.3 mg K^+ g^{-1} clay, respectively. The lower K^+-fixing capacity (=1.3 mg K^+ g^{-1} clay) of Trebur soil owing to lower CEC (80-100 meq/100g), lower layer charge (0.2-0.4) and lower K^+ selectivity of smectite clay minerals . Moreover, soil Trebur was dominant in smectite (50%) (Paul, 1989), which had less K^+ fixation due to its less attraction for K^+ ions. Instead of showing K^+-fixing capacity by conventional methods, such soils may release K^+ to fulfill the plant requirements (Wakeel, 2010, 2011).

Therefore it is concluded that the clay minerals present in the soils should be considered while conducting research experiments and recommending K^+ fertilizers. In most part of the developing world, K^+ fertilizers are recommended generally without considering clay minerals present in the soil and recommended fertilization do not give appropriate results discouraging the farmers to use K^+ fertilizers for crop production. We suggest the K^+ fertilizer recommendation considering soil clay minerals and also suggest that EUF may be a good tool to determine K^+-dynamics in the soils varying in clay contents.

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Emir. J. Food Agric. 2013. 25 (1): 45-51
doi: 10.9755/ejfa.v25i1.4937
http://www.ejfa.info/

PLANT SCIENCE

Morphological diversity of eggplant (*Solanum melongena*) in Bangladesh

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Abstract

Morphological diversity in 92 eggplant genotypes based on twenty one characters was estimated using Mahalanobis's D^2 statistics. The highest intra-cluster distance was observed in cluster VIII (2.13), containing seven genotypes and the lowest intra-cluster distance (1.18) was observed in cluster IV having four genotypes. Ninety two eggplant genotypes were grouped into ten different clusters by non-hierarchical clustering. The cluster X had the maximum number (17) of genotypes and cluster II and III had minimum number (3) of genotypes. The highest inter-cluster distance was observed between cluster II and VIII (30.86) indicated the genotypes in these clusters were more diverged than those of other clusters. The lowest inter-cluster distance was observed between the clusters V and X (3.72) suggesting a close relationship among the genotypes included within these clusters. Cluster II constitute three genotypes and produced the highest mean value for number of flowers per inflorescence (4.67) and yield per plant (812.33) and the lowest mean value days to 1st flowering (108.22). Cluster IV constitute three genotypes namely EP-080, EP-081, EP-089 and produced fruits for longer duration (82.33). Cluster VIII constitute seven genotypes and showed the lowest mean value for number of infected shoots per plant (1.57). Cluster X established with 17 genotypes produced the lowest mean value for number of infected fruit per plant (8.26). Therefore, more emphasis should be given on cluster II, IV and VIII for selecting genotypes as parents for crossing which may produce new recombinants with desired traits.

Key words: Eggplant, Genotypes, Genetic divergence, Multivariate, Cluster analysis, D^2 statistics

Introduction

Brinjal or eggplant or aubergine (*Solanum melongena* L.) is indigenous to a vast area stretching from northeast India and Burma, to Northern Thailand, Laos, Vietnam and Southwest China and wild plants can still be found in these locations (Daunay and Janick, 2007). Eggplant was domesticated from wild forms in the Indo-Burma region with indications that it was cultivated in antiquity. Several Sanskrit documents, dated from as early as 300 BCE, mention this plant with various descriptive words, which suggest its wide popularity as food and medicine (Nadkarni, 1927). In the *Ayurvedic*, a Hindi system of medicine, white types were recommended for diabetic patients, and roots for the treatment of asthma (Khan, 1979). Eggplant is widely cultivated as vegetable in both temperate and tropical areas, especially in Asia. Eggplant is a major fruit vegetable with world production exceeding 32 million tonnes (Mt). The world leading eggplant producers are China (18.2 Mt), India (15.6 Mt), Egypt (2.0 Mt), Turkey (1.3 Mt), Indonesia (0.7 Mt), Iraq (0.6 Mt) Japan (0.6 Mt) and Italy (0.5 Mt each) (FAO, 2008). Eggplant is particularly favoured in Asia where it has been cultivated for millennia, and in India it is considered King of Vegetables.

For an effective breeding program, information concerning the extent and nature of genetic diversity within a crop species is essential. It is particularly useful for characterizing individual accessions and cultivars and as a general guide in the selection of the parents for hybridization (Furini and Wunder, 2004). Better knowledge on genetic diversity or genetic similarity could help to sustain long term selection gain (Chowdhury et al., 2002). Improvement in yield and quality is normally

Received 25 January 2011; Revised 18 October 2011; Accepted 26 October 2011; Published Online 24 November 2012

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achieved by selecting genotypes with desirable character combinations existing in the nature or by hybridization. Selection of parents identified on the basis of divergence analysis would be more promising for a hybridization program. Some related results have been reported in eggplant (Tambe et al., 1993; Chaudhary and Pathania, 1998; Singh and Gapalakrishnan, 1999; Kumar et al., 2000). A previous knowledge of the structure of the genetic diversity within a large collection of germplasm may be of great help to make decisions on management procedures, as well as on breeding strategies to use in current and future breeding programs. According to Sharma and Jana (2002), assessment of genetic variation in a species is a prerequisite for initiating an efficient breeding program, as it provides a basis for tailoring desirable genotypes. Genetically diverse parents are likely to segregates and or to produce high heterotic crosses. More diverse the parents, greater are the chances of obtaining high heterotic F_1s and broad spectrum of variability in segregating generations (Arunachalam, 1981). Genetic diversity study also permits to select the genetically divergent parents to obtain the desirable recombinant in the segregating generations of eggplants. The aim of the present study was to characterize eggplant genotypes collected from different regions of Bangladesh and exotic sources to assess the genetic diversity within the germplasm. Assessment of genetic diversity is important for selecting breeding strategies.

Materials and Methods

Ninety two genotypes of indigenous and exotic eggplants collected from the Plant Genetic Resources Centre (PGRC) and Horticulture Research Centre (HRC) of Bangladesh Agricultural Research Institute (BARI) were studied to measure the diversity among the genotypes at the Field Laboratory, Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur. Genotypes of eggplant representing samples from different origins (local and exotic from AVRDC). The seeds of these germplasm were sown on the seedbed and thirty five days old seedlings were transplanted in the main field. The experiment was laid out in a randomized complete block design with three replications. The unit plot size was 7.5 × 0.70m and 10 plants were accommodated in a plot with a plant spacing of 75cm maintaining a row distance of 70cm. Data were recorded from five randomly selected plants from each plot for leaf blade length (cm), leaf blade width (cm), leaf blade lobing, leaf blade tip angle, leaf prickles, leaf blade color, petiol color, days to 1st flowering, number of flowers per inflorescence, plant height at flowering stage (cm), plant breadth at flowering stage (cm), number of primary branches per plant, days to 1st fruit setting, fruit length (cm), fruit breath (cm), fruit length / breath ratio, days to 1st fruit harvest, duration of fruiting (days), number of healthy shoots per plant, number of infected shoots per plant, yield per plant (g). The selection of morphological characters was made by applying the IBPGR eggplant descriptor (IBPGR 1990). Plot means over the replications were used for the statistical analysis. Genetic diversity was studied following Mahalanobis's (1936) generalized distance (D^2) extended by Rao (1952). Based on the D^2 values, the genotypes were grouped into clusters following the method suggested by Tocher (Rao, 1952; Jager et al., 1983; Digby et al., 1989). Intra and inter cluster distances were calculated by the methods of Singh and Chaudhury (1985). Statistical analyses were carried out using GENSTAT 5 software.

Results and Discussion

D^2 Statistics

Analysis of variance exhibited significant differences among the genotypes under study. Thus, it indicated notable genetic variability among the 92 genotypes.

Principal component analysis (PCA)

Analysis yielded eigen values of each principle component axes of coordination of genotype with the first axes totally accounted for 20.07% variation among the genotype, while eight of these eigen values above unity accounted for 73.87% of the total variation among the 21 principal component axes describing 92 genotypes of eggplant (Table 1).

Principal coordinate analysis (PCO)

The inter genotypic distances (D^2) as obtained by principle coordinate analysis (PCO) for all possible combinations between the pairs of genotypes. Inter genotypic distances as obtained from principle coordinate analysis showed that the highest distance was observed between the genotype EP-047 and EP-039 followed by the genotype E-074 and EP-047 (Table 2). The lowest distance was observed between genotype EP-026 and EP-025. The difference between the highest and the lowest inter genotypic distance indicated the prevalence of variability among the 92 genotypes of eggplant studied.

Intra-cluster distance were calculated from these inter genotypic distances (Singh and Chaudhury, 1985). The highest intra-cluster distance was observed in cluster VIII (2.13), containing seven genotypes, followed by cluster III (2.05) containing

11 genotypes. The lowest intra-cluster distance (1.18) was observed in cluster IV having four genotypes. The intra-cluster distances in all the ten clusters were lower than the inter-cluster distances and which indicated that genotypes within the same cluster were closely related.

Non-hierarchical clustering

With the application of co-variance matrix for non-hierarchical clustering, 92 eggplant genotypes were grouped into ten different clusters. The clustering pattern obtained coincided with the apparent grouping patterns performed by PCA. So the results obtained through PCA were confirmed by non-hierarchical clustering. Table 3 represents the clusters occupied by 92 genotypes of eggplant. The cluster X had the maximum (17) number of genotypes, followed by cluster VI (15 genotypes). The cluster I and cluster III had 11 genotypes, cluster IX had 10 genotypes, cluster V had eight genotypes, cluster VII and cluster VIII had 7 genotypes, c luster II and III had 3 genotypes.

These results confirmed the clustering pattern of the genotype according to the principle component analysis. Composition of different cluster with their corresponding genotypes and collection site included in each cluster are presented in table. The cluster pattern obtained from the nonhierarchical clustering is coincidence with the apparent grouping pattern perform by PCA. For the reason it can be said that the results obtained through PCA were established by non-hierarchical clustering.

Table 1. Eigen values and percentage of variation for corresponding 21 component characters in 92 genotypes of eggplant.

Principle component axes	Eigen values	Percentage of total variation	Percentage of cumulative
A	4.21	20.07	20.07
B	3.03	14.43	34.5
C	2.00	9.54	44.04
D	1.49	7.12	51.16
E	1.41	6.75	57.91
F	1.28	6.11	64.02
G	1.06	5.06	69.08
H	1.00	4.79	73.87
I	0.82	3.94	77.81
J	0.69	3.30	81.11
K	0.68	3.25	84.36
L	0.60	2.90	87.26
M	0.52	2.48	89.74
N	0.48	2.30	92.04
O	0.39	1.86	93.9
P	0.33	1.61	95.51
Q	0.26	1.27	96.78
R	0.24	1.18	97.96
S	0.19	0.93	98.89
T	0.14	0.69	99.58
U	0.09	0.44	100.00

Table 2. Five highest and five lowest inter genotypic distances of eggplant genotypes.

Genotype	Distance	Genotype	Distance
Highest value		Lowest value	
EP-047 – EP-039	4.058	EP-026 – EP-025	0.598
EP-074 – EP-047	3.965	EP-089 – EP-034	0.646
EP-079 – EP-047	9.904	EP-064 – EP-034	0.669
EP-086 – EP-030	3.875	EP-089 – EP-087	0.689
EP-047 – EP-008	3.820	EP-003– EP-002	0.696

Table 3. Distribution of 92 genotypes of eggplant in different cluster.

Cluster number	Number of genotypes	Genotypes
I	11	EP-019, EP-024, EP-033, EP-037, EP-043, EP-049, EP-051, EP-053, EP-074, EP-087, EP-088
II	3	EP-017, EP-039, EP-079
III	11	EP 12, EP 13, EP 15, EP 20, EP 23, EP 27, EP 46, EP 50, EP 57, EP 86, EP 90
IV	3	EP 80, EP 81, EP 89
V	8	EP3, EP8, EP18, EP31, EP59, EP68, EP77, EP 91
VI	15	EP14, EP16, EP29, EP35, EP41, EP45, EP56, EP 61, EP65, EP66, EP67, EP69, EP73, EP75, EP 92
VII	7	EP 22, EP 30, EP 34, EP 36, EP 44, EP 64, EP 70
VIII	7	EP 11, EP 21, EP 47, EP 48, EP 54, EP 62, EP 76
IX	10	EP 1, EP 2, EP 6, EP 25, EP 38, EP 42, EP 52, EP 60, EP 71, EP 84
X	17	EP-004, EP-005, EP-007, EP-009, EP-010, EP-026, EP-028, EP-032, EP-040, EP-055, EP-058, EP-063, EP-072, EP-078, EP-082, EP-083, EP-085

Table 4. Intra and inter cluster distances among 92 genotypes of eggplant.

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X
I	1.74	8.107	16.293	11.446	7.881	19.048	10.069	23.356	13.395	4.801
II		1.64	23.829	19.205	15.605	26.498	17.673	30.876	20.940	12.474
III			2.05	5.867	9.114	3.914	6.821	7.366	3.844	11.688
IV				1.18	4.978	8.696	3.909	12.668	4.027	7.058
V					1.97	12.004	4.261	16.011	6.088	3.727
VI						1.96	9.374	5.379	6.606	14.505
VII							1.90	13.706	4.605	5.631
VIII								2.13	10.430	18.775
IX									1.99	8.853
X										2.00

Canonical variate analysis

Canonical variate analysis was done to compute the inter-cluster Mahalanobis's D^2 values. The intra and inter cluster distance (D^2) value are presented in Table 4. Result indicated that the highest inter-cluster distance was observed between cluster II and VIII (30.86) followed by cluster II and VI (26.49) and cluster II and III (23.82). The maximum inter-cluster distances between these clusters indicate wide spectrum of variability of population. However, the highest inter-cluster distance was observed between cluster II and VIII indicated the genotypes in these clusters were more diverged than those of other clusters. The lowest inter-cluster distance was observed between the clusters V and X (3.72), suggesting a close relationship among the genotypes included within these clusters. The intra-cluster distance varied from 1.13 to 2.13, the maximum for cluster VIII that was composed seven genotypes of diverse origin, while the minimum distance was found in cluster IV that was composed three genotypes. Statistical distances represent the index of genetic diversity among the cluster. The inter-cluster distances were larger than the intra-cluster distances that indicated wider genetic diversity among the genotypes of different groups.

Cluster mean value

The individual genotype was characterized in respect of their mean values for different character with a view to get idea that weather genotypes having similar characteristics could be disseminated. The mean values for all the 21 characters along with the marking of the highest (H) and the lowest (L) for each of the cluster are presented in Table 5. Cluster II constitute three genotypes and produced the highest mean value for number of flowers per inflorescence (4.67), number of infected shoot per plant (8.67), yield per plant (812.33). But the lowest mean value leaf blade width (4.33), leaf blade lobbing (4.33), leaf blade color (3.00), days to 1st flowering (108.22). Cluster III constitute 11 genotypes namely EP-012, EP-013, EP-015, EP-020, EP-023, EP-027, EP-046, EP-050, EP-057, EP-086, EP-090 produce the

highest mean value for fruit length breath ratio (5.82). Cluster IV constitute three genotypes namely EP-080, EP-081, EP-089 and produced the highest plant height at flowering stage (35.47), plant breath at flowering stage (56.86), fruit length (14.50), duration of fruiting (82.33). Cluster V constitute eight genotypes namely EP-003, EP-008, EP-018, EP-031, EP-059, EP-068, EP-077, EP-091 produce the highest mean value for leaf blade length (5.50), leaf blade width (6.00), fruit breadth (6.53). Cluster VIII constitute seven genotypes namely EP-011, EP-021, EP-047, EP-048, EP-054, EP-062, EP-076 produce the highest mean value for days to 1st flowering (129.12), number of primary branches per plant (6.29) But the lowest mean value duration of fruiting (54.98), number of infected shoot per plant (1.57),yield per plant (121.00). Cluster X established with 17 genotypes namely EP-004, EP-005, EP-007, EP-009, EP-010, EP-026, EP-028, EP-032, EP-040, EP-055, EP-058, EP-063, EP-072, EP-078, EP-082, EP-083, EP-085 produced the lowest mean value for number of infected fruit per plant (8.26).

Table 5. Cluster mean values of 21 characters of 92 genotypes in brinjal.

Character	Cluster									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Leaf blade length	5.18	4.33(L)	5.00	5.00	5.50(H)	4.87	5.29	5.00	5.40	4.88
Leaf blade width	5.36	4.33(L)	5.18	5.67	6.00(H)	5.53	5.57	5.29	5.60	5.47
Leaf blade lobing	4.73	4.33(L)	5.18	5.00	5.00	5.40	5.29	4.43	5.20	5.24(H)
Leaf blade tip angle	4.64	4.33	4.45	5.00(H)	4.75	4.87	4.43	4.43	4.40	4.29(L)
Leaf prickles	0.55	0.33	0.64	0.00	1.37	1.07	1.00	1.43	1.40	1.53
Leaf blade color	6.27	3.00	5.36	7.00	6.00	5.00	5.00	5.57	5.40	4.88
Petiol color	2.55	2.00	1.91	2.67	2.87	2.07	2.00	1.86	3.60	2.06
Days to 1st flowering	117.05	108.22	124.26	126.00	123.04	126.43	128.59	129.12	120.35	122.31
No. of flowers per inflorescence	3.45	4.67	3.37	2.00	3.62	3.36	2.86	3.14	3.80	3.00
Plant height at flowering stage	30.47	29.77	27.03	35.47	28.80	38.85	33.43	28.78	31.25	31.04
Plant breadth at flowering stage	47.79	41.70	47.72	56.86	48.58	50.60	52.85	50.89	47.48	48.80
No. of primary branches per plant	5.82	5.33	5.82	4.67	5.50	5.67	5.86	6.29	5.50	5.41
Days to 1st fruit setting	133.42	121.11	136.11	134.68	139.60	138.46	144.59	143.34	133.71	136.54
Fruit length	10.33	9.68	13.31	14.50	9.95	12.33	10.09	11.76	10.07	9.74
Fruit breath	4.23	3.14	3.29	3.02	6.53	3.90	4.27	3.71	3.98	4.55
Fruit length / breath ratio	3.03	3.00	5.82	5.07	2.01	4.45	2.99	4.74	2.38	2.86
Days to 1st fruit harvest	187.14	182.22	187.73	182.11	182.17	203.94	200.40	195.36	182.17	187.86
Duration of fruiting	76.91	80.83	72.03	82.33	75.71	57.51	63.76	54.98	76.03	74.53
No of healthy shoot per plant	8.59	9.56	9.61	8.67	6.67	9.45	10.21	8.67	9.33	8.26
No of infected shoot per plant	5.76	8.67	3.36	2.89	3.87	1.93	2.81	1.57	2.93	4.00
Yield per plant	643.51	812.33	288.39	395.67	476.17	229.39	426.55	121.00	354.92	540.08

Table 6. Latent vectors for 21 component characters of 92 genotype of eggplant.

Characters	Vector 1	Vector 2	Characters	Vector 1	Vector 2
Leaf blade length	0.03	-0.12	No. of primary branches per plant	0.08	0.14
Leaf blade width	0.24	-0.24	Days to 1st fruit setting	0.38	0.07
Leaf blade lobing	0.14	-0.07	Fruit length	-0.01	0.41
Leaf blade tip angle	0.08	-0.08	Fruit breadth	0.18	-0.35
Leaf prickles	0.12	-0.26	Fruit length / breath ratio	-0.05	0.47
Leaf blade color	-0.13	0.28	Days to 1st fruit harvest	0.31	0.18
Petiole color	-0.12	0.11	Duration of fruiting	-0.34	-0.22
Days to 1st flowering	0.36	0.13	No of healthy shoots per plant	-0.02	0.07
No. of flowers per inflorescence	-0.20	0.18	No of infected shoot per plant	-0.36	-0.02
Plant height at flowering stage	0.11	0.08	Yield per plant	-0.24	-0.23
Plant breadth at flowering stage	0.24	0.01			

Contribution of characters toward divergence of the genotypes

Contribution of characters towards the divergence obtained from canonical variate analysis is presented in Table 6. In this method vectors was calculated to represent the varieties in the graphical form (Rao, 1952). This is helpful in cluster analysis as it facilitates the study of group constellations and also serves as a pictorial representation of the configuration of various groups. The absolute magnitude of the coefficients in the first two canonical vectors also reflects to a great extent, the importance of the characters for primary and secondary differentiation. The character, which gives high absolute magnitude for vector 1, is considered to be responsible for primary differentiation. Likewise, the characters, which give higher absolute magnitude for vector 2 is considered to be responsible for secondary differentiation. If the same character gives equal magnitude for both the vectors then the character is considered responsible for primary as well as secondary differentiation. The PCA revealed that the important characters responsible for genetic divergence in the major axis of differentiation (Vector 1) were leaf blade width, days to 1st flowering, plant breath at flowering stage, days to 1st fruit setting, fruit breadth, and days to 1st fruit harvest (Table 6). It was the reflection of first axis of differentiation. In vector 2, leaf blade color, days to 1st flowering, plant breath at flowering stage, plant height at flowering stage, no of flower per inflorescence, number of primary branches per plant, fruit length, days to 1st fruit harvest showed their important role towards genetic divergence. Other characters played a minor role in determining genetic divergence. The role of days to 1st fruit harvest, days to 1st flowering for both the vectors was positive across two axes indicating the important components of genetic divergence in these materials.

Selection of genotypes as parent for hybridization program

The inter cluster distance between II and VIII was the highest followed by II- VI (26.498), II- III (23.829) and I- VIII (23.356). Cluster distance between cluster I- III, I- VI, II- IV, II- V, II- VII, II- IX, V- VIII, VI- X and VIII- X were moderate or intermediate. Intermediate diverse parents have the more chance to contribute heterosis in the subsequent generations. Arunachalan *et al*. (1984) suggested in groundnut that the magnitude of heterosis for yield and its components were found higher in crosses between inter mediate divergences than extreme ones. Ramanujam *et al*. (1974) suggested in mung bean that there was a fair agreement between the extent of heterosis and the genetic divergence between parents. To select cluster for more heterotic genotypes four pairs of clusters II & VIII, II & VI, II & III and I & VIII could be considered.

Cluster II had the highest cluster mean for number of flowers per inflorescence, yield per plant and the lowest for days to first flowering. The cluster IV had the highest cluster mean for Plant breadth at first flowering, fruit length and duration of flowering. Cluster V had the highest cluster mean for fruit breadth and the lowest for number of infested shoots per plant. Cluster VIII had the highest cluster mean for number of primary branches per plant and the lowest for number of infested fruits per plant. So, hybridization between the genotypes of cluster II, IV, V and VIII will manifest maximum heterosis and create wide genetic variability for economic characters in eggplant. Considering cluster distance and cluster mean, the genotypes EP- 017 and EP- 039 from cluster II, genotypes EP- 089 from cluster IV,

genotypes EP- 003 and EP- 091 from cluster V, genotypes EP- 011, EP- 021, EP- 047 and EP- 054 from cluster VIII may be considered as parents for future hybridization program.

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Emir. J. Food Agric. 2013. 25 (1): 52-57
doi: 10.9755/ejfa.v25i1.15396
http://www.ejfa.info/

PLANT SCIENCE

Agronomic performance of mash bean as an intercrop in sesame under different planting patterns

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Abstract

In a field study, the performance of mashbean in a sesame-mash intercropping system under different geometric arrangements was evaluated on a sandy-clay loam soil at the University of Agriculture, Faisalabad for two consecutive years. The planting pattern comprised 40 cm spaced single rows, 60 cm spaced 2-row strips and 100 cm spaced 4-row strips while mashbean was intercropped in all the three planting patterns and also grown as sole crop. The results revealed that planting sesame in the pattern of 100 cm spaced 4-row strips explored the possibility of intercropping mashbean in sesame by producing significantly maximum seed yield of mashbean (703.10 kg ha^{-1}) and base crop i.e. sesame (687.40 kg ha^{-1}) as compared to 40 cm spaced single rows and 60 cm spaced 2-row strips planting patterns. Besides, the intercropping sesame with mashbean in pattern of 100 cm spaced 4-row strips appeared to be feasible, convenient and gave maximum net profit (Rs.21242 ha^{-1}) as compared to other two planting patterns and monocropped sesame.

Key words: Agronomic performance, Planting patterns, Intercropping, Sesame

Introduction

The role of legumes as a source of vegetable protein and their beneficial effects on residual soil fertility is well known. Unfortunately, these crops being grown on poor soils of rain fed areas could not benefit from the impact of green revolution, which resulted in chronic low yield and shortage of pulses in country for the last many years. At present the gross domestic production of pulses in Pakistan is 937.30 thousand tones with net availability of 680.68 kg ha^{-1} $annum^{-1}$. Pakistan spent 92.2 million dollars on import of pulses to meet the growing need of her population (Anonymous, 2012) by spending huge amount of foreign exchange.

In view of shrinkage resources particularly arable land, area, irrigation water and energy, the only option left is to increase the production per unit area/time. Of the possible agronomic approaches to meet this challenge, an intercropping technology is an immediate option since it as been a proven technology for the last many years in Indo-Pak subcontinent and is now showing commercial prospects (Jabbar et al., 2005; Ahmad et al., 2007; Jabbar et al., 2011). In the past, monocropping of grain legumes (pulses) was a usual practice among the growers but now-a-days the interest in growing food legumes as intercrops is increasing. Recent evidence suggests that there are remarkable benefits of legumes intercropping which are achieved not only by means of costly inputs but by the simple expedient of growing crops together in an appropriate geometry (Bhatti at el., 2008). When legumes are grown in association with non-legumes, there is often advantage to the non-legumes from nitrogen fixed by the legumes (Wahla et al., 2009). Besides, two crops differing in height, canopy, adaptation and growth habits grow simultaneously with least competition (Jabbar et al., 2009). Other suggested forms of advantages are the greater stability of yield over different seasons, better use of land resources, possibility of better control of weeds, pests and diseases (Jabbar et al., 2010a,b).

Received 09 February 2011; Revised 14 July 2011; Accepted 26 August 2011; Published Online 24 November 2012

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Pakistan is a subtropical country having adequate irrigation and land resources with high intensity of sunlight for plant growth. Therefore, possibility of raising two or more crops on the same piece of land in a year needs to be explored for effective and efficient utilization of these natural resources. Intercropping is an efficient and most economical production system as it not only increases the production per unit area and time but also improves the resource-use efficiency and economic standard of the growers. Presently, interest in intercropping is emerging among the small growers because of their diversified needs and low farm income from the monocropping system.

So in the present scenario of preponderance of small holding, surplus farm family labour, overlapping of growing season of crops, low productivity of most of the crops and practice of subsistence farming intercropping seems to be a promising strategy for increasing crop productivity particularly at small farm level in Pakistan.

The conventional method of planting sesame in 40-cm spaced single rows, however, does not permit intercropping because of narrow inter-row spacing. Recently a new method of planting sesame in well-spaced multi-row strips has been developed which not only gives relatively higher seed yield than the conventional single row planting (Nazir et al. (2001) and Jabbar et al. (2011) but also facilitates intercropping, harvesting and handling of the intercrops without doing damage to the base crop.

In Pakistan, no systematic research seems to have been done so far to explore the possibility of intercropping of various legume crops in sesame. Thus, there is a need to develop an appropriate sesame-legume intercropping system. The present study was, therefore, planned to explore the feasibility and production potential of mashbean (*Vigna mungo* L.) as intercrop in sesame under different planting patterns in irrigated environment at Faisalabad.

Materials and Methods

The study was conducted at the agronomic research area, University of Agriculture, Faisalabad on a sandy clay loam soil. The planting patterns comprised 40 cm spaced single rows, 60 cm spaced 2-row strips and 100 cm spaced 4-row strips of sesame while mashbean was intercropped in all the three planting patterns and also grown as a sole crop. Mashbean was intercropped in sesame on the same day on the vacant space between the sesame strips. The plant population was kept constant and optimum in all the three geometric arrangements. The experiment was laid out in a randomized complete block design with four replications. The net plot size was 3.2 m x 7.0 m. A basal dose of 50-100-50 kg NPK ha^{-1} was applied at the time of sowing while additional 50 kg N ha^{-1} was applied with first irrigation only to the sesame crop to meet its full nitrogen requirement. In all three irrigations each of 7.5 cm were given to mature the component crops. The first irrigation was given 20 days after germination and the second 35 days after germination while the third one at flowering. Both the sesame and mashbean were harvested at their physiological maturity. The crops were harvested manually at the ground level with the help of a sickle, dried in the sun light and then tied into separate bundles. Both the crops were threshed manually. The observations were recorded on the desired parameters as per treatment by using standard procedures. The data collected were statistically analyzed by using the computer statistical programme "MSTAT-C (Freed and Eisensmith, 1986) and Fisher's analysis of variance technique (Steel et al., 1997) and the Least Significant Differences (LSD) test at P=0.05 was used to compare the differences among treatment means.

Results and discussion

Seed yield (kg ha^{-1})

The 2-year average data revealed that mash bean grown as sole crop produced significantly higher seed yield (808.40 kg ha^{-1}) than that obtained as intercrop in all the three planting patterns (Table 1).

Within the planting patterns, mashbean intercropped in sesame planted in the pattern of 100 cm spaced 4-row strips produced significantly higher seed yield (703.1. kg ha^{-1}) than that intercropped in 60 cm spaced paired rows (621.1 kg ha^{-1}) compared to the minimum (503.70 kg ha^{-1}) when intercropped in sesame planted in 40 cm spaced ingle rows. Yield reduction in the intercropped mashbean was attributed to less number of branches and pods $plant^{-1}$, less seeds $plant^{-1}$ and lower 1000-seed weight compared to the sole crop of mashbean (Table 1). Suppressive effect of intercropping on different yield components of mashbean grown in association with sesame might be due to variable inter-specific competition for different growth factors. This competition was not severe in case of mashbean intercropped in sesame planted in 100 cm spaced 4-row strips as compared to its intercropping in 60 cm spaced single rows. The former planting pattern promoted more light

and air penetration. Khan and Khaliq (2004) also reported a significant reduction in seed yield of soybeean intercropped in cotton. Similarly Bhatti et al. (2008) also observed significant reduction in seed yield of mungbean due to associated culture in sesame.

Stalk yield (kg ha^{-1})

Mashbean grown as a sole crop produced significantly higher stalk yield (2538.00 kg ha^{-1}) than that realized from mashbean as intercrop in all the three planting patterns (Table 1). However, stalk yield of mashbean intercropped in 100 cm spaced 4-row strips of sesame was significantly higher (2332.00 kg ha^{-1}) than that intercropped in 60 cm spaced paired rows (2133.00 kg ha^{-1}). By contrast, the minimum stalk yield of mashbean was recorded when intercropped in sesame planted in 40 cm spaced single rows (1834.00 kg ha^{-1}).

Biological yield (kg ha^{-1})

Intercropping had a significant effect on the biological yield of mashbean intercrops. However, mashbean grown alone in 40 spaced rows produced significantly higher biological yield (3335.00 kg ha^{-1}) than that grown as intercrop under different planting patterns (Table 1).

Among the planting patterns, mashbean intercropped in sesame planted in the pattern of 100 cm spaced 4-row strips gave significantly higher biological yield (3036.00 kg ha^{-1}) than that intercropped in the pattern of 60 cm spaced paired rows (2755 kg ha^{-1}) against the minimum (2337 kg ha^{-1}) when mashbean was intercropped in sesame planted in 40 cm spaced single rows. The variation in biological yield of mashbean intercropped in different planting patterns was attributed to dominant effect of the sesame which suppressed the vegetative growth of mashbean. Similar reduction in biological yield of soybean was reported by Khan and Khaliq (2004) when intercropped in cotton. Similarly Bhatti et al. (2008) also observed significant reduction in biological yield of mungbean when intercropped with sesame.

Number of branches plant^{-1}

Effect of intercropping on branches plant^{-1} of mashbean was significant. Mashbean grown alone in 40 cm spaced rows produced significantly greater number of branches plant^{-1} (6.28) that that grown as intercrop in different planting patterns (Table 1).

Among the planting patterns, mashbean intercropped in sesame grown as intercrop in the pattern of 100 cm spaced 4-row strips produced significantly more branches plant^{-1} (5.07) than that intercropped in 60 cm spaced paired rows (4.64) compared to the minimum (4.15) when mashbean was intercropped in sesame planted in 40 cm spaced single rows. Significantly less number of branches in intercropped mashbean was attributed to competition between the component crops for growth factors and dominant behaviour of sesame. Similar results were reported by Bhatti et al. (2008) when mungbean was intercropped with sesame. Khan and Khaliq (2004) also reported significant effect on branches plant^{-1} of soybean when intercropped in cotton.

Number of pods plant^{-1}

The intercropping effect on pods plant^{-1} of mashbean was significant. The average across two years showed two years showed that sole crop produced significantly greater number of pods plant^{-1} (27.97) than that grown as intercrop under different geometric arrangements. As regards the planting patterns, mashbean intercropped in sesame grown under the pattern of 100 cm spaced 4-row strips produced significantly greater number of pods plant^{-1} (25.98) than that intercropped under the pattern of 60 cm spaced paired rows (24.17). By contrast, the minimum pods plant^{-1} (22.19) was recorded when mashbean was intercropped in sesame planted in 40 cm spaced single rows. Khan and Khaliq (2004) and Bhatti et al. (2008) also reported similar findings when soybean was intercropped with cotton and mungbean intercropped with sesame.

Number of seeds pod^{-1}

Mashbean intercropped in sesame irrespective to geometric arrangement or grown as sole crop produced statistically similar number of seeds pod^{-1} as recorded for sole crop which on an average ranged between 5.25 and 5.40.

Number of seeds plant^{-1}

There were significant differences among different planting patterns with regard to seeds plant^{-1}. Monocropped mashbean produced significantly greater number of seeds plant^{-1} (144.10) than that grown as intercrops under different planting patterns. Within the planting pattern, mashbean intercropped in sesame grown in the pattern of 100 cm spaced 4-row strips produced significantly greater number of seeds plant^{-1} (132.30) than that intercropped in 60 cm spaced paired rows (121.20). Contrarily, the minimum seeds plant^{-1} were recorded (105.10) when mashbean was intercropped in sesame planted in 40 cm spaced single rows. The variation in seeds plant^{-1} under different planting patterns was

ascribed to inter-species competition and dominant effect of sesame. Khan and Khaliq (2004) also reported variation in the number of seeds plant^{-1} of soybean under different intercropping systems. Bhatti et al. (2008) also favoured these results when mungbean was intercropped in sesame.

Seed weight plant^{-1}

Effect of intercropping on seed weight plant^{-1} of mashbean was significant. The crop grown alone in 40 cm spaced rows produced significantly higher seed weight plant^{-1} (5.17 g) than that grown as intercrop under different planting patterns. Among the planting patterns, mashbean intercropped in sesame grown in the pattern of 100 cm spaced 4-row strips produced significantly higher seed weight plant^{-1} (4.12 g). By contrast, the minimum seed weight plant^{-1} (3.26 g) was recorded when mashbean was intercropped in sesame planted in 40 cm spaced single rows.

1000-seed weight (g)

The crop grown alone in 40 cm spaced single rows or intercropped in the pattern of 100 cm spaced 4-row strips exhibited statistically similar 1000-seed weight of 37.04 and 36.18 g, respectively while the minimum 1000-seed weight (32.50 g) was recorded when mashbean was intercropped in sesame planted in 40 cm spaced single rows. These results are in line with the findings of Bhatti et al. (2008). On the contrary, Khan and Khaliq (2004) reported non-significant effect on 1000-seed weight of soybean when intercropped in cotton.

Plant density (m^{-2}) at harvest

Mashbean intercropped in all the three planting patterns had statistically equal plant density m^{-2} which was at par with that recorded for the sole crop of mashbean. However, the plant density m^{-2} on an average ranged between 25.76 to 26.21 m^{-2}. These results are in line with the findings of Bhatti et al. (2008) who intercropped mungbean in sesame but not in agreement with those reported by Khan and Khaliq (2004) who found a significant reduction in plant population of soybean when intercropped in cotton.

Plant height (cm)

The crop grown alone in 40 cm spaced rows attained significantly more plant height (56.31 cm) than that grown as intercrops under different planting patterns. Within the planting patterns, mashbean intercropped in sesame planted in the pattern of 100 cm spaced 4-row strips attained significantly greater plant height (53.96 cm) than that intercropped in the pattern of 60 cm spaced paired rows (51.89 cm) against the minimum (51.00 cm) when mashbean was intercropped in sesame planted in 40 cm spaced single rows. Bhatti et al. (2008) also reported significant effect on plant height of mungbean when intercropped in sesame. On the contrary, Khan and Khaliq (2004) reported non-significant effect on plant height of soybean when intercropped in cotton.

Table 1. Agro-economic performance of mashbean intercropped in sesame under different planting patterns.

Parameters	40 cm spaced single rows (P1)	60 cm spaced double rows (P2)	100 cm spaced 4-row strips (P3)	Mashbean alone (P4)	LSD
Seed yield of base crop (sesame) (kg ha^{-1})	518.05 c	646.55 b	687.40 a	-	21.86
Seed yield (kg ha^{-1})	503.7 d	621.2 c	703.10 b	808.4 a	33.32
Stalk yield (kg ha^{-1})	1834 d	2133 c	2332 b	2538 a	117.60
Biological yield (kg ha^{-1})	2337 d	2755 c	3036 b	3345 a	149.90
Branches plant^{-1}	4.15 d	4.64 c	5.07 b	6.28 a	0.22
Pods plant^{-1}	22.19 d	24.17 d	25.98 b	27.97 a	1.11
Seeds pod^{-1}	5.25	5.29	5.38	5.40	NS
Seeds plant^{-1}	105.1 d	121.2 c	132.3 b	144.1 a	3.14
Seed weight plant^{-1}	3.26 d	4.12 c	4.92 b	5.17 a	0.16
1000-seed weight (g)	32.50 c	34.07 b	36.18 a	37.04 a	0.89
Plant density m^{-2}	25.76	25.89	26.14	26.21	NS
Plant height (cm)	51.00 d	51.89 c	53.96 b	56.31 a	0.71
Harvest index (%)	21.45 d	22.55 c	23.16 b	24.17 a	0.19
Seed protein (%)	23.86	24.54	24.86	25.05	NS
Net field benefits (Rs ha^{-1})	13353	19443	21242	16866	

Any two means not sharing a letter differ significantly at 5% level of probability (LSD); NS = Non-significant

Table 2. Economic analysis of intercropping mash bean with sesame at different geometric arrangements.

Gross field benefits (Rs. ha^{-1})		
Geometric arrangements	Sesame alone	Sesame + mashbean
P_1= 40 cm spaced single rows	20053	18755
P_2= 60 cm spaced paired rows	19198	24843
P_3= 100 cm spaced 4-row strips	19806	26643
Total cost that vary (Rs. ha^{-1})		
P_1= 40 cm spaced single rows	3120	5402
P_2= 60 cm spaced paired rows	2220	5400
P_3= 100 cm spaced 4-row strips	3120	5400
Net field benefits (Rs. ha^{-1})		
P1= 40 cm spaced single rows	16933	13353
P2= 60 cm spaced paired rows	16978	19443
P3= 100 cm spaced 4-row strips	16686	21242

Harvest index (%)

The sole crop of mashbean exhibited significantly higher harvest index (24.17%) than that recorded for mashbean intercropped in different geometric arrangements. Among the planting patterns, mashbean intercropped in sesame grown in the pattern of 100 cm spaced 4-row strips gave significantly higher harvest index (23.16 %) than that intercropped in the pattern of 60 cm spaced paired rows (22.55%). However, the minimum (21.45 %) harvest index was recorded when mashbean was intercropped in sesame planted in 40 cm spaced single rows. Reduction in harvest indices as a result of intercropping was also reported by Bhatti et al. (2008) when mungbean was intercropped in sesame but Khan and Khaliq (2004) reported non-significant effect on H.I when soybean was sown in association with cotton.

Crude seed protein concentration (%)

The protein concentration in mashbean seed of a sole crop was statistically equal to that intercropped in sesame under all the three geometric arrangements. The seed protein concentration, however, on an average ranged between 23.86 and 25.05 %. These results are in line with those of Bhatti et al. (2008).

Economic analysis

The net benefits of intercropping mashbean in the pattern of 100 cm spaced 4-row strips or 60 cm spaced paired rows of sesame was higher (Rs.21242 ha^{-1}) than that achieved from planting sesame at 60 cm spaced paired rows (19443) and 40 cm spaced single rows (13353) (Table 2). Hence, it is recommended that small farmers with limited resources may adopt the practice of sesame + mashbean intercropping preferably in the form of 100 cm spaced 4-row strips planting system of sesame.

Conclusion

It is concluded that intercropping of mashbean in sesame grown in the pattern of 100 cm spaced 4-row strips is more economical and remunerative than intercropping of mashbean in sesame in the pattern of 40 cm spaced single rows and 60 cm spaced double rows.

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Emir. J. Food Agric. 2013. 25 (1): 58-65
doi: 10.9755/ejfa.v25i1.9666
http://www.ejfa.info/

ANIMAL SCIENCE

Effect of in-ovo administration with two levels of amino acids mixture on the performance of Muscovy ducks

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Abstract

Nutrient administration in-ovo could be considered as an alternative method to improve hatchability and duckling weight followed by better economic performance. On the 12th day of incubation, fertile duck eggs (n= 500) were distributed into 5 groups, each of 100. These were: un-injected control; 0.50 ml distilled water; 0.50 ml amino acids (AA) mixture; 0.75 ml distilled water; and 0.75 ml amino acids mixture. In-ovo injection of 0.50 ml but not 0.75 ml of AA mixture resulted in higher hatchability percentage than un-injected control; however this was not statistically confirmed. In-ovo injection of either 0.50 or 0.75 ml of AA mixture resulted in significantly ($P < 0.05$) higher body weight at hatch, marketing weight for males not for females, and higher feed intake than the un-injected control. There was no significant difference ($P < 0.05$) in feed conversion ratio between in-ovo amino acids ducks and un-injected control during the whole experimental periods. Liver weight as a percentage of body weight was higher ($P < 0.05$) in the in-ovo amino acids injected groups than un-injected control. Lymphoid organs of 0.50 AA-injected male group and 0.75 ml AA-injected female group were significantly ($P < 0.05$) heavier than the un-injected control. Antibodies titers did not differ ($P < 0.05$) between in-ovo amino acids injected groups and un-injected control. It is concluded that in-ovo injection of amino acids mixture may improve and accelerate growth and post-hatch performance of Muscovy ducks.

Key words: Amino acids, In-ovo injection, Muscovy duck, Performance

Introduction

There is a linear improvement in the growth performance and meat yield of commercial poultry with better input efficiency each year (Havenstein et al., 2003; Ferket, 2004). Different factors play crucial roles in influencing hatchability and growth performance during embryonic and post-hatch life, for instance genetic, egg characteristics and incubation environment (Narushin and Romanov, 2002; Petwket et al., 2003; Abiola et al., 2008). Nutrients utilization in the egg's embryo is crucial and their transfer from the mother to her embryo is completed before the egg is laid, thus the egg contains all of nutrients needed for the growth and development of the embryo. Hatched chicks are affected by the nutrients in the yolk remaining in the peritoneal cavity post-hatching (Romanoff, 1960). Nowadays, in ovo-injection is widely used for many purposes, such as fertilizing an avian egg in the shell (Cantrell and Wooten, 2003), injecting avian eggs with immunological material (Jochemsen and Jeurissen, 2002), a trial for sex reversal in birds (Kagmi and Hanada, 1997), increasing the post-hatching body weights of birds by in-ovo injection of growth promoters (Ohta et al., 1999), and enhancing the growth of avian embryo by injecting eggs with special liquid nutritional supplements. Nutrients in-ovo injection has a lot of benefits: greater efficiency of feed utilization (Bhanja et al., 2004); reduced post-hatch mortality and morbidity; improved immune response (Gore and Qureshi, 1997); enhanced early growth by improving intestinal function and development (Tako et al., 2004); and increased skeletal growth (Hargis et al., 1989), breast muscle yield (Hajihosaini and Mottaghitalab, 2004), and marketing body weight (Selim et al., 2012).

Broiler breeder eggs contain an excess of fat and moisture while protein store may be limited (Al-Murrani, 1978), and yolk protein is the origin of the required amino acids (AA) during

Received 19 December 2011; Revised 13 February 2012; Accepted 21 February 2012; Published Online 24 November 2012

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embryogenesis (Gerhartz et al., 1999). Therefore, embryonic and post-embryonic performance may be improved by in-ovo injection of amino acids (Al- Murrani, 1982), and their derivatives either sporadic or in mixture. Amino acids were injected in the fertile eggs of poultry either single amino acid such as arginine and threonine or amino acids metabolites as β-hydroxy-ß-methyl butyrate (a leucine metabolite) or as mixture of essential and/or non-essential amino acids which mostly identical to the amino acids profile of egg protein (pattern relative to lysine). However, researches concerning in-ovo nutrients administration are minimal. Owing to the importance of in-ovo injection and its role in improving the hatchling weight, the objective of this research was to assess the impact of in-ovo injection of two levels of amino acids mixture (0.50 and 0.75 ml) on post-hatch growth, carcass traits, lymphoid organs and antibody titer in Muscovy ducks.

Materials and Methods

Eggs incubation and injection

Standard size fertile duck eggs (n= 500) were collected from Muscovy breeders ducks fed on adequate nutritional diet. Fertility was verified by candling with a hand ultraviolet lamp at day 12 of incubation, Eggs were distributed into five groups of 100 eggs each. Eggs were incubated at 37 to 37.5ºC and 70% relative humidity during the first 32 days of incubation. Eggs were turned automatically every hour until the 32nd day. All eggs were transferred to the hatchery at the end of the 32nd day of incubation, and placed in hatching boxes at 37°C temperature and 70-75 % relative humidity until hatching occurred between days 35 and 36. On the 12th day of incubation, the groups of fertile eggs were distributed into five groups: uninjected control; 0.50 ml amino acids mixture (Aminoleban®; Egypt Otsuka Pharmaceutical Company); 0.50 ml distilled water (D.W); 0.75 ml amino acids mixture; and 0.75 ml distilled water. The treatment solutions or sham control were injected into the yolk of the 12-day-old embryo which was identified by candling with a hand ultraviolet lamp, through a pinhole made at the broad end of the egg, using a 25 mm needle. Prior to in-ovo injection the injection site was disinfected with 70% ethanol and the solutions were warmed to 30°C. The pinhole site was sealed with sterile paraffin wax immediately after injection. The injected eggs were returned to the incubator after injection. This amino acids mixture is of identical pattern to the egg's amino acids, which was calculated as percentage of lysine content of the egg. Where, the ideal ratio of amino acids to lysine remains largely unaffected by dietary, environmental and genetic factors (Schutte and Jong, 2004). The composition of amino acids solution was shown in Table 1.

Ducklings and housing

All hatched ducklings from each treatment were weighed and sexed to males and females subgroups. The ducklings in each treatment group either males or females were randomly assigned to 4 replicates depending upon the hatch size, each replicate with a pen in each treatment. All ducklings were reared under similar managerial and hygienic conditions. The ducklings were raised in clean, well-ventilated, previously disinfected room. A continuous lighting program was maintained throughout the nine weeks experimental period. Temperature was adjusted at 32ºC ± 2 in the first week and then reduced by 2ºC each successive week then maintained at 22ºC ± 2. The ducklings of all groups were vaccinated once against Avian Influenza with 0.50 ml single dose of Reassortant H5N1 Avian Influenza vaccine (Re-1 vaccine) subcutaneously in the lower back of the neck at the 14th day old (Middleton et al., 2007). Ducklings of different experimental groups fed on basal starter (0-3 weeks), grower (3-6 weeks) and finisher (6-9 weeks) rations as shown in Table 2. Basal rations were formulated to meet the nutrients requirements for Muscovy ducks as recommended by French Group Company, Sadat City, Egypt (Strain' origin). Nutrients compositions of the used rations were calculated according to the feed composition tables given by NRC (1994). The ducklings were fed ad libitum on dry mash ration and fresh clean water was constantly available.

Table 1, Composition of injected amino acids* solution into fertile eggs.

L-Amino acids	Content (mg/ml)	% of lysine
Arginine HCL	7.3	96.05
Histidine HCL	3.2	42.10
Methionine	1.0	13.20
Phenylalanine	1.0	13.20
Threonine	4.5	59.20
Valine	8.4	110.50
Lysine HCL	7.6	100.00
Tryptophan	7.0	92.47
Leucine	11.0	144.70
Isoleucine	9.0	118.40
Proline	9.0	118.40
Serine	5.0	66.12
Alanine	7.5	99.16
Cysteine HCL	4.0	52.90

* Identical to egg's amino acids composition.

Growth parameters

After hatching, the body weights (BW) of the ducklings in each group were recorded. The body weight of individual ducklings in each group and feed consumption of each pen were recorded at 3, 6 and 9 weeks of age. The feed conversion ratio (FCR) was calculated accordingly.

Sampling

Blood samples were taken from tibial vein of eight birds in each group (4 males and 4 females) at 5 and 9 week of age by needle under aseptic precaution. Separation of serum was carried out by centrifugation of coagulated blood at 3000 rpm for 15 minutes for determination of antibodies titer against Avian Influenza disease virus by Haemagglutination Inhibition test (Thayer and Beard, 1998). At the end of the experimental period (9 weeks of age) 8 birds (4 males and 4 females) were selected from each treatment group and were slaughtered after being fasted for 12 hours. After slaughter and complete bleeding, the birds were dressed. The carcass and some other components (liver, gizzard, abdominal fat, heart, bursa of Fabricius, spleen, and thymus) were weighed.

Dressing percentage = [(Dressed carcass weight/Live body weight) × 100].

Relative organ weights were calculated as percentages of body weight = [(Organ weight/Body weight) × 100].

Total edible parts were calculated as percentage of body weight = [(Weight of liver + gizzard + heart + abdominal fat)/Body weight) × 100].

Statistical Analysis

The obtained data were presented as means ± SE. Analysis of variance (ANOVA) was used to test the significance of the difference between different treatments and statistical differences were established using a Duncan's Multiple Range Test (Duncan, 1955) at the level of $P < 0.05$.

Results and Discussion

Research has been shown that embryos survived after in-ovo injection of amino acids into the yolk (Al-Murrani, 1982) with little impacts on hatchability percentage (Hajihosaini and Mottaghitalab, 2004; Ohta et al., 1999 and 2001). In-ovo injection of 0.50 ml (84%) but not 0.75 ml of AA mixture (68%) into the yolk of fertilized Muscovy duck's eggs at the 12th day of incubation increased hatchability percentage compared to the un-injected control (74%) and D.W injected groups (72%). However, this was not statistically confirmed. This increase in hatchability percentage after in-ovo injection of 0.50 ml of AA might be due to the timing for injection, during the period of rapid growth of embryo and/or the necessary amount of amino acids administered into fertilized eggs.

Table 2. Composition and nutritional levels of experimental diets.

Ingredients (%)	Ration		
	Starter	Grower	Finisher
Ground yellow corn	61.40	67.00	68.10
Soya bean meal (44 % CP)	30.00	23.40	20.00
Corn gluten meal (60 % CP)	4.30	5.00	5.00
Vegetable oil[1]	0.40	0.70	3.00
Mono calcium phosphate[2]	1.36	1.42	1.36
Ground limestone	1.73	1.72	1.54
Salt (NaCl)	0.31	0.30	0.40
Mineral and Vitamin Premix[3]	0.30	0.30	0.40
L-Lysine[4]	0.10	0.10	0.15
L-Methionine[5]	0.10	0.10	0.10
Calculated composition			
ME MJ/Kg diet	12.2	12.6	13.1
Crude protein (g/kg)	211.0	191.0	177.0
Calcium (g/kg)	9.7	9.6	8.7
Available phosphorus (g/kg)	4.2	4.2	4.0
Lysine (g/kg)	10.8	9.3	8.8
Methionine (g/kg)	4.6	4.4	4.2
Methionine + Cystine (g/kg)	8.1	7.7	7.3

1Vegetable oil composed of a mixture of soybean, cotton seed and sunflower oils.

2Monocalcium phosphate; 21% phosphorus, and 15% calcium.

3Minerals and Vitamins mixture (Pharma Mix). Each 3 kg contain: 12.000.000 I.U. Vitamin A, 2.500.000 I.U. Vitamin D3, 10.000 mg Vitamin E, 2.00 mg Vitamin K3, 1.000 mg Vitamin B1, 5.000 mg Vitamin B2, 1.500 mg Vitamin B6, 10 mg Vitamin B12, 30.000 mg, Niacin 1.000 mg, Folic acid, 50 mg Biotin, 10.000 mg Pantothenic acid, 10.000 mg Copper, 10.000 mg Iodine, 100 mg Selenium, 30.000 mg Iron, 60.000 mg Manganese, 50.000 mg Zinc, 100 mg Cobalt, CaCo3 add to 3000 gm; 4L-lysine: 78% produced by Archar Daniels Medland Company De Caur I.L. made in U.S.A. (ADM); 5DL-methionine: 99% Canadian registration number 990137 Guaranteed analysis, DL-Methionine 99%.

These results are in consistent with Ohta and Kidd (2001) who noted that the percentage of hatchability increased by in-ovo administration of 0.50 ml of AA mixture into the yolk of broiler eggs at the 7th day and at the 14th day of incubation in the work of Bhanja and Mandal (2005). Gaafar (2009) found that in-ovo injection of 0.50 ml of amino acids mixture into the yolk of fertilized Muscovy duck's eggs at day 12th of incubation increased hatchability percentage as compared to the control group. On the other hand, in-ovo injection of nutrients may provide an alternative method to increasing duckling weight at hatch. In-ovo injection of 0.50 ml (55.05 g) or 0.75 ml (52.84 g) of amino acids mixture significantly increased ($P < 0.05$) body weights of ducklings at hatch compared to the uninjected control (45.95 g). On the other hand, there was no significant difference ($P < 0.05$) between the uninjected control and sham groups (47.36 g and 49.45 g for 0.50 ml and 0.75 ml distilled water injected groups, respectively). This increase in ducklings weights might be due to the increased available free amino acids into eggs, that are not normally in excess amount, where the embryo development is restricted to the nutrients content of eggs (Klein, 1968; Rupe and Farmer, 1955) and are excessively utilized during the period from days 7 to 14 of incubation (Ohta and Sato, 2005).

In-ovo injection of identical AA mixture improved post-hatching growth and marketing weight as a result of increasing available AA remaining in the peritoneal cavity post-hatching (Romanoff, 1960), which might be enhanced the protein synthesis after hatching. In-ovo injection of 0.50 or 0.75 ml of AA significantly increased ($P < 0.05$) body weights of males than the un-injected control during the experimental periods. Moreover, there was no significant difference in body weights between in-ovo amino acids females and un-injected control except during the starting period for 0.50 ml and starting and growing periods for 0.75 ml; it significantly increased (Table 3). These results were in agreement with Al-Murrani (1982) who recorded that injection of broiler eggs with 0.50 ml of AA increased body weight by 12.7 % at 56 days of age, and Gaafar (2009) recorded that body weights significantly increased in male and female ducks hatched from eggs injected with amino acids mixture compared with the control group. Foye et al. (2006) reported that in-ovo feeding of 0.7 % Arginine and β-hydroxy-β-methylbutyrate resulted in a 27.5 g higher body weight at 14 days of age. Furthermore, Bhanja and Mandal (2005) reported that in-ovo injection of specific amino acids (Ile + leu + Val) or (Gly + Pro) resulted in 63.2-63.6 g higher body weight at 3rd week of age. In another study, Bakyaraj et al. (2012) found that in ovo feeding of AA for CMI response (lysine, methionine, arginine, leucine, and isoleucine) resulted in significantly higher ($P < 0.01$) body weight at 21 days of age than the sham control group. In the present study, a higher body weight was recorded in the AA groups where arginine, leucine, isoleucine, methionine, lysine, valine and proline were constituents in the in-ovo amino acids mixture solution.

Table 3. Body weight (g) of male and female Muscovy ducklings hatched from eggs injected with two levels of amino acids mixture in different growth periods (mean ± SE).

Group Period	Control	AA (0.50 ml)	D. W (0.50 ml)	AA (0.75 ml)	D. W (0.75 ml)
Male					
Starting	551.6 ± 12.8^{b}	829.7 ± 8.0^{a}	549.4 ± 3.0^{b}	820.0 ± 7.1^{a}	553.0 ± 4.0^{b}
Growing	2196.6 ± 41.2^{c}	2483.6 ± 23.9^{b}	2173.7 ± 10.7^{c}	2608.6 ± 10.5^{a}	2200.0 ± 19.8^{c}
Finishing	4109.2 ± 55.2^{c}	4565.0 ± 31.2^{a}	4055.3 ± 44.0^{c}	4350.0 ± 11.5^{b}	4150.0 ± 14.6^{d}
Female					
Starting	572.0 ± 8.1^{b}	651.0 ± 6.9^{a}	569.2 ± 4.8^{b}	644.7 ± 8.5^{a}	572.8 ± 2.4^{b}
Growing	1770.5 ± 27.7^{b}	1800.1 ± 12.7^{b}	1772.5 ± 11.5^{b}	1916.5 ± 12.0^{a}	1776.7 ± 8.5^{b}
Finishing	2801.9 ± 32.6^{a}	2731.7 ± 56.9^{a}	2765.0 ± 16.1^{a}	2778.1 ± 20.1^{a}	2766.7 ± 15.3^{a}

a-b Means within the same row having different superscript are significantly different ($P < 0.05$). AA = Amino Acids mixture, D.W = Distilled Water

Table 4. Feed conversion ratio (FCR) of male and female Muscovy ducklings hatched from eggs injected with two levels of amino acids mixture in different growth periods (mean ± SE).

Group Period	Control	AA (0.50 ml)	D. W (0.50 ml)	AA (0.75 ml)	D. W (0.75 ml)
Male					
Starting	1.47 ± 0.04[a]	1.49 ± 0.01[a]	1.48 ± 0.02[a]	1.48 ± 0.01[a]	1.47 ± 0.01[a]
Growing	1.82 ± 0.04[a]	1.79 ± 0.02[a]	1.78 ± 0.04[a]	1.78 ± 0.01[a]	1.79 ± 0.04[a]
Finishing	2.21 ± 0.04[a]	2.28 ± 0.03[a]	2.14 ± 0.09[a]	2.25 ± 0.01[a]	2.11 ± 0.05[a]
Female					
Starting	1.40 ± 0.02[a]	1.40 ± 0.01[a]	1.38 ± 0.02[a]	1.36 ± 0.01[a]	1.38 ± 0.01[a]
Growing	2.25 ± 0.04[a]	2.21 ± 0.01[a]	2.23 ± 0.01[a]	2.28 ± 0.03[a]	2.26 ± 0.01[a]
Finishing	3.30 ± 0.06[a]	3.32 ± 0.05[a]	3.38 ± 0.05[a]	3.39 ± 0.07[a]	3.36 ± 0.03[a]

a-b Means within the same row having different superscript are significantly different (P < 0.05). AA = Amino Acids mixture, D.W = Distilled Water

In-ovo injection with 0.50 or 0.75 ml of AA resulted in an increase in the feed intake of male and female ducks as compared to the un-injected control group (data not shown). Corresponding higher body weight was also found in those groups. This higher feed intake of ducklings hatched from eggs injected with amino acids mixture may attribute to greater body weight as feed intake is a function of live body weight wherein all the treatment groups had similar diets. Therefore, the ducklings which had higher body weight must have consumed higher feed, provided there was no difference in FCR (Table 4). These results were in agreement with Bhanja and Mandal (2005) who reported that higher feed intake in the amino acid-injected groups than the control group. Bhanja et al. (2004) recorded that there was no difference in feed intake and FCR, but apparently better FCR was found in AA-injected chicks. In another study, Kadam et al. (2008) reported that feed conversion until 17 day after hatching was improved by in-ovo injection of 10, 20 or 30 mg of threonine. Moreover, Bakyaraj et al. (2012) reported that in ovo injection of AA for CMI response resulted in significantly higher feed intake with no variation in FCR during the 3-week experiment than the untreated control.

There have been substantial studies looking at improving carcass traits. In-ovo injection of nutrients is interested possible method to meet this objective, but there is very little research looking at in-ovo injection with amino acids. In-ovo injection of 0.50 or 0.75 ml of AA caused no significant changes (P < 0.05) in the dressing percentage of male ducks but increased in females group injected with 0.50 ml of AA mixture as compared to un-injected control. On the other hand, In-ovo injection of either 0.50 or 0.75 ml of AA mixture resulted in significant increase (P < 0.05) in the dressing percentage compared to the sham group. In-ovo injection of amino acids mixture resulted in significant increase (P < 0.05) in the liver weight for male and female ducks compared to the un-injected control and sham groups (Table 5).

Table 5. Carcass traits of male and female Muscovy ducks after in-ovo injection with amino acids mixture (mean ± SE):

Group Item(as % of BW)	Control	AA (0.50 ml)	D. W (0.50 ml)	AA (0.75 ml)	D. W (0.75 ml)
Male					
Dressing	82.5 ± 1.1[a]	80.9 ± 0.8[a]	77.0 ± 1.0[b]	82.1 ± 0.2[a]	75.8 ± 0.2[b]
Liver	2.15 ± 0.12[b]	2.47 ± 0.01[a]	2.00 ± 0.01[b]	2.66 ± 0.20[a]	1.85 ± 0.08[b]
Gizzard	2.52 ± 0.08[a]	2.53 ± 0.12[a]	2.24 ± 0.02[a]	2.31 ± 0.06[a]	2.51 ± 0.13[a]
Heart	0.67 ± 0.01[a]	0.65 ± 0.01[a]	0.68 ± 0.01[a]	0.70 ± 0.01[a]	0.69 ± 0.01[a]
Abdominal fat	2.30 ± 0.30[a]	2.03 ± 0.30[a]	2.30 ± 0.40[a]	2.68 ± 0.22[a]	2.30 ± 0.22[a]
Total edible parts	7.64 ± 0.54[a]	7.68 ± 0.16[a]	7.22 ± 0.12[a]	8.35 ± 0.10[a]	7.35 ± 0.16[a]
Female					
Dressing	75.7 ± 0.1[b]	80.1 ± 0.4[a]	72.3 ± 0.4[c]	75.9 ± 1.2[b]	72.6 ± 0.4[c]
Liver	1.92 ± 0.02[c]	2.41 ± 0.01[a]	1.83 ± 0.04[c]	2.09 ± 0.01[b]	1.80 ± 0.03[c]
Gizzard	2.33 ± 0.02[a]	2.23 ± 0.01[a]	2.35 ± 0.03[a]	2.27 ± 0.01[a]	2.35 ± 0.03[a]
Heart	0.77 ± 0.01[a]	0.76 ± 0.01[a]	0.71 ± 0.05[a]	0.78 ± 0.01[a]	0.71 ± 0.04[a]
Abdominal fat	2.64 ± 0.30[a]	2.41 ± 0.40[a]	2.74 ± 0.20[a]	2.37 ± 0.37[a]	2.50 ± 0.36[a]
Total edible parts	7.66 ± 0.12[a]	7.81 ± 0.03[a]	7.81 ± 0.27[a]	7.51 ± 0.05[a]	7.36 ± 0.34[a]

a,b Means within the same row having different superscript are significantly different (P < 0.05). AA = Amino Acids mixture, D.W = Distilled Water

Table 6. Lymphoid organ weight of male and female Muscovy ducks after in-ovo injection with amino acids mixture (Means ± SE).

Group Item	Control	AA (0.50 ml)	D. water (0.50 ml)	AA (0.75 ml)	D. water (0.75 ml)
Male					
Thymus	0.545 ± 0.006^{b}	0.555 ± 0.022^{b}	0.480 ± 0.004^{c}	0.715 ± 0.010^{a}	0.495 ± 0.010^{c}
Bursa	0.131 ± 0.004^{c}	0.145 ± 0.006^{bc}	0.155 ± 0.002^{b}	0.200 ± 0.012^{a}	0.145 ± 0.006^{bc}
Spleen	0.054 ± 0.001^{c}	0.069 ± 0.001^{b}	0.044 ± 0.001^{d}	0.080 ± 0.002^{a}	0.035 ± 0.002^{d}
Female					
Thymus	0.620 ± 0.016^{b}	0.712 ± 0.010^{a}	0.455 ± 0.022^{c}	0.655 ± 0.006^{b}	0.462 ± 0.025^{c}
Bursa	0.118 ± 0.007^{b}	0.200 ± 0.012^{a}	0.135 ± 0.002^{b}	0.132 ± 0.001^{b}	0.128 ± 0.014^{b}
Spleen	0.051 ± 0.001^{b}	0.075 ± 0.001^{a}	0.061 ± 0.003^{ab}	0.060 ± 0.004^{ab}	0.057 ± 0.006^{b}

a,b Means within the same row having different superscript are significantly different ($P < 0.05$). AA = Amino Acids mixture, D.W = Distilled Water

Table 7. Antibody titers (HI titer log – 2)* of male and female Muscovy ducks after in-ovo injection with amino acids mixture (mean ± SE).

Group Item	Control	AA (0.50 ml)	D. W (0.50 ml)	AA (0.75 ml)	D. W (0.75 ml)
Male					
1st antibody titer	3.5 ± 0.2^{a}	3.0 ± 0.4^{a}	3.0 ± 0.2^{a}	3.5 ± 0.2^{a}	3.0 ± 0.3^{a}
2nd antibody titer	3.0 ± 0.4^{a}	3.5 ± 0.2^{a}	3.0 ± 0.4^{a}	3.5 ± 0.2^{a}	3.0 ± 0.4^{a}
Female					
1st antibody titer	3.5 ± 0.2^{a}	3.5 ± 0.2^{a}	3.0 ± 0.2^{a}	3.5 ± 0.2^{a}	3.0 ± 0.2^{a}
2nd antibody titer	3.5 ± 0.2^{a}	3.5 ± 0.2^{a}	3.0 ± 0.5^{a}	4.0 ± 0.4^{a}	3.0 ± 0.6^{a}

a,b Means within the same row having different superscript are significantly different ($P < 0.05$). AA = Amino Acids mixture, D.W = Distilled Water. * Against H5N1 reassortant Avian Influenza vaccine

Total edible parts % of 0.50 or 0.75 ml AA injected males and females were not affected as compared to the control group. These results were nearly similar to the results of Bhanja et al. (2004) who observed that broiler's carcass characteristics and cut-up parts yield at 6 weeks of age did not vary between AA injected and control birds, and Johri (2004) recorded that in-ovo injection of AA did not affect the digestive organs and carcass yield. However, Hajihosaini and Mottaghitalab (2004) found that complete and edible carcass weights were higher in broiler obtained from egg treated with AA injected in yolk sac than in the control group.

The relative weights of thymus gland and bursa of fabricus were comparatively higher in 0.50 ml AA injected males group; however the relative weight of spleen significantly increased ($P < 0.05$) as compared to the control. Moreover, in-ovo injected females had higher lymphoid organ weights ($P < 0.05$) than un-injected control and sham group. On the other hand, in-ovo injection of 0.75 ml of amino acids mixture significantly increased ($P < 0.05$) lymphoid organ weights in male ducks only as compared to the un-injected control and sham group (Table 6). These results were partially agree with Bhanja et al. (2004) who showed that there were no significant differences in the weights of immune organs in the AA injected birds and the control group. However, the weights of thymus and spleen were comparatively higher in AA injected group than the control birds when in-ovo injections were carried out on the 14th day of incubation.

In-ovo injection of fertile eggs with 0.50 or 0.75 ml of AA mixture resulted in no significant changes in the geometric means of the 1st and 2nd estimates for antibodies titers of male and female groups as compared to the control group (Table 7). These results were similar to those of Hajihosaini and Mottaghitalab (2004) who found that no significant difference between the AA injected group and the control one in terms of antibodies titer.

Conclusion

Early supplementation of nutrients through in-ovo injection such as amino acids mixture can be regarded as a possible method to improve hatchability, body weight at hatch, marketing weights, and immune status of Muscovy ducks. Further investigations are needed to highlight the effect of in-ovo injection of amino acids mixture on the humoral, cell mediated immunity, and development of digestive organs in Muscovy ducks.

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Emir. J. Food Agric. 2013. 25 (1): 66-80
doi: 10.9755/ejfa.v25i1.9138
http://www.ejfa.info/

ANIMAL SCIENCE

A review on role of exogenous enzyme supplementation in poultry production

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Abstract

Monogastric animals like poultry, pigs, etc. lack the alloenzymes from rumen microflora and thus it become necessary to incorporate the enzymes in their diets in order to derive optimal nutrient utilization from complex feed matric. Feed enzymes are added to animal feed to increase the availability of nutrient by digesting the feed components during storage or after consumption within the gastrointestinal tract. Some of the enzymes that have been used over the past several years and have potential for use in the feed industry include cellulase (β-glucanases), xylanases and associated enzymes, phytases, proteases, lipases, and galactosidases. Most of the enzymes used in the feed industry have been applied for poultry to neutralize the effects of the viscous, non-starch polysaccharides in cereals such as barley, wheat, rye, and triticale. Recently, considerable interest has been shown in the use of phytase as a feed additive. The addition of phytase to feed improves phosphorus utilization in both ruminant and monogastric animals, reducing the need for supplemental inorganic phosphate and helps in reducing the environmental problems that arise from organic phosphate excretion. Thus, enzyme supplementation in the feed play an important role in increasing the availability of nutrients and retarding the adverse effect of anti-nutritional factors present in the feed components. This review aims to elucidate the different enzymes being used in the poultry industry and their efficiency in increasing the poultry production.

Key words: Enzyme, Poultry nutrition, Growth, Phytase, Phosphate excretion

Introduction

The application of feed enzymes to poultry diets for the enhancement of nutrient availability had been reported since 1926. Previously, the research conducted on feed enzymes in poultry nutrition focused on non-starch polysaccharide (NSP) degrading enzymes, especially xylanase and β-glucanase, in diets containing wheat, rye and barley (Choct, 2006). The use of unconventional feedstuff for poultry production is however limited due to their fibrousness and inability of birds to possess the cellulase enzyme that can digest the fibre (Adebiyi et al., 2010). Exogenous enzymes capable of degrading non-starch polysaccharides (NSP) in broiler diets based on 'viscous' grains, including wheat and barley (Bedford and Schulze, 1998), initiated this practice. Non-starch polysaccharides mostly present in raw materials used for poultry diets are pectins, cellulose, mixed-linked β-glucans and arabinoxylans (Parsippany, 2008). Depolymerisation of these NSPs requires specific enzymes, these enzymes are specific to the main and side chain structure of the NSP. The first development and use of NSP-degrading enzymes was applied in barley and then in wheat based broiler diets. Researchers found that the use of NSP-degrading enzymes in barley and wheat based broiler diets improved litter quality and performance. The use of NSP-degrading enzymes in wheat and barley based diets for broilers are therefore well established and accepted (Bedford, 2009).

The animal rearing practice depends on the application of feeds that are uniform in quality and have high nutritive value. The complex plant materials that are commonly used as feed ingredients, such as coarsely processed grains and high-fiber feedstuffs (e.g., cereal grains, forages,

Received 02 December 2011; Revised 09 July 2012; Accepted 21 August 2012; Published Online 24 November 2012

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and crop residues) have nutritive components that are resistant to endogenously-produced digestive enzymes. Some feed components also have anti-nutritive effects, for example phytate, which reduces bioavailability of certain minerals, and oligosaccharides and other soluble carbohydrates that increase viscosity and reduce nutrient absorption. Hence, obtaining the maximal nutritive value from such complex feedstuffs typically requires supplementation of autoenzymatic activity with alloenzymatic activity (i.e., exogenously produced digestive enzymes from non-host sources) (Klasing, 1998). Feed enzymes are typically added to animal feed to increase availability of nutrient by acting on feed components prior to or after consumption, i.e., within the gastrointestinal tract (Pariza and Cook, 2010). The effect of dietary enzyme on the animal is influenced by the type and concentration of the undesirable carbohydrate present in the feedstuff and the class and age of the livestock and poultry that consume it. Young chicks are affected to a greater degree by anti-nutritional compounds than older birds (Marquardt et al., 1996). Enzymes that appear to be beneficial for non-ruminant animals are the xylanases, or more specifically the endoxylanases for the feed which contain wheat, triticale and rye and the β-glucanases or cellulases for those which contain barley and oats (Marquardt et al., 1996). Most commercial enzymes contain a spectrum of different enzymes including xylanases and β-glucanases and therefore can be used effectively with the above cereals. It is, nevertheless, essential to ensure that the enzyme preparation has the appropriate activities of the specific enzymes that are required. Phytase, in addition to the above mentioned enzymes, is an enzyme, which increases the availability of phosphorus from phytate, a bound form of phosphate found in cereals and other plant material (Marquardt et al., 1996). It has become available for use in the feed industry and may assist in reducing phosphorus requirements in non-ruminant animals and therefore it can solve problems associated with environmental pollution. During the past decade, the inclusion of microbial phytase in poultry diets has increased drastically with proved success, mainly in response to heightened concerns over phosphorus pollution of the environment.

While supplementing the poultry feed with enzymes, it is important to consider several factors which are as follows (Marquardt et al., 1996):

1. The enzyme supplement must contain the proper spectrum of enzymes, so that anti-nutritive effects of target substrate will be neutralized.
2. Different cereals contain different amounts of the enzyme-sensitive anti-nutritional factor. Therefore, the response to enzyme treatment may vary within a given cereal (i.e. barley and probably wheat).
3. Outcomes of the enzyme supplementation are affected by grade and age of poultry. The responses in swine are usually less dramatic than those of poultry and have not been clearly established.
4. The activity of the enzymes must not be affected by processing or by the low pH (<4) or digestive enzymes in the gastrointestinal tract.

Ruminant animals (e.g., cattle and sheep) have the advantage of alloenzymatic digestion provided by rumen microflora, which enables ruminants to obtain nutrients from complex feed matrices that are not made available through autoenzymatic digestion (Pariza and Cook, 2010). Pigs, poultry and other monogastric animals lack the alloenzymes from rumen microflora, so for these species to derive optimal nutrient benefit from complex feed matrices, it is necessary to provide added enzyme supplementation. Enzymes may act during feed processing, while feed is present in storage and feeder bins, and also following the ingestion by acting within the digestive system itself (Pariza and Cook, 2010). Moreover, the addition of enzyme like phytase has also been known to improves phosphorus utilization in both ruminant (Kincaid et al., 2005; Knowlton et al., 2007) and monogastric animals (Nnenna et al., 2006), thus, reducing the need for supplemental inorganic phosphate and the environmental problems that arise from organic phosphate excretion. Therefore, the main objective of exogenous enzyme supplementations is to improve feed efficiency for monogastric animals like poultry.

Various enzymes currently used in poultry feed

The exploitation of microbial enzymes at industrial level started 100 years ago with the patenting of a process for the production of alpha-amylase from the fungus *Aspergillus oryzae* (Pariza and Cook, 2010). Most of the enzymes currently used in the food and beverage industry are from *Aspergillus*, however, hemicellulases and cellulases are derived from *Trichoderma.* Some of the enzymes that have been used over the past several years or have potential for use in the feed industry include cellulase (β-glucanases), xylanases and associated enzymes, phytases, proteases, lipases,

and galactosidases (Table 1). For instance the use of combination of xylanase and β-glucanase has been proved to be beneficial in terms of growth increment in the poultry. Pettersson and Aman (2007) tested the addition of an enzyme cocktail, consisting of xylanase and β-glucanase to an unpelleted poultry diet containing rye and wheat. The enzyme cocktail was added at various inclusion levels. The addition of the enzyme cocktail in Pettersson and Aman's (2007) trail resulted in a significant increase in body-weight and feed intake.

Table 1. Enzymes currently used in poultry feed.

Enzyme Name	Classification	Production organism	Function
α-Amylase	Carbohydrase	*Aspergillus spp., Bacillus spp., Rhizopus spp.*	Hydrolyzes starch
Maltogenic a-amylase	Carbohydrase	*Bacillus subtilis, d-Bacillus stearothermophilus*.	Hydrolyzes starch with production of maltose
β-Amylase	Carbohydrase	Barley malt	Hydrolyzes starch with production of maltose
Cellulase	Carbohydrase	*Aspergillus niger.*	Breaks down cellulose
α-Galactosidase	Carbohydrase	*Aspergillus niger, Morteirella vinaceae var Saccharomyces sp.*	Hydrolyzes oligosaccharides
β-Glucanase	Carbohydrases	*Aspergillus spp., Bacillus spp.*	Hydrolyzes B-glucans
β-Glucosidase	Carbohydrase	*Aspergillus niger*	Hydrolyzes cellulose degradation products to glucose
Glucoamylase (amylo-glucosidase)	Carbohydrase	*Aspergillus niger, Aspergillus oryzae, Rhizopus niveus, Rhizopus oryzae.*	Hydrolyzes starch with production of glucose
Hemicellulase	Carbohydrase	*Aspergillus spp., Bacillus spp., Humicola sp., Trichoderma sp.*	Breaks down hemicellulose
Invertase	Carbohydrase	*Aspergillus niger, Saccharomyces sp.*	Hydrolyzes sucrose to glucose and fructose
Lactase	Carbohydrase	*Aspergillus niger, Aspergillus oryzae, Candida pseudotropicalis, Kluyveromyces marxianis.*	Hydrolyzes lactose to glucose and galactose
β-Mannanase	Carbohydrase	*Aspergillus niger, Bacillus lentus, Trichoderma reeseic*	Hydrolyzes beta-mannans
Pectinase	Carbohydrase	*Aspergillus aculeatus, Aspergillus niger, Rhizopus oryzae*	Breaks down pectin
Pullulanase	Carbohydrase	*Bacillus acidopullulyticus*	Hydrolyzes starch
Xylanase	Carbohydrase	*Aspergillus spp., Bacillus spp., Humicola sp., Penicillum sp., Trichoderma sp.*	Hydrolyzes xylans
Lipase	Lipase	*Aspergillus niger, Candida sp., Rhizomucor sp., Rhizopus sp.,*	Hydrolyze triglycerides, diglycerides, and glycerol monoesters
Bromelain	Protease	Pineapple (*Ananas comosus*) stem and fruit	Hydrolyzes proteins
Ficain	Protease	*Ficus glabrata*	Hydrolyzes proteins
Keratinase	Protease	*Bacillus licheniformis*	Hydrolyzes proteins
Papain	Protease	Papaya (*Carica papaya*)	Hydrolyzes proteins
Pepsin	Protease	Animal stomachs	Hydrolyzes proteins
Protease	Protease	*Aspergillus niger, Aspergillus sp., Bacillus spp.*	Hydrolyzes proteins
Trypsin	Protease	Animal pancreas	Hydrolyzes proteins
Catalase	Oxidoreductase	*Aspergillus niger var., Micrococcus lysodeikticus*	Produces water and oxygen from hydrogen peroxide
Glucose Oxidase	Oxidoreductase	*Aspergillus niger*	Degrades glucose to hydrogen peroxide and gluconic acid
Phytase	Phosphatase	*Aspergillus spp., Penicillium sp., Phytase canola, Pichia pastoris, d-Aspergillus niger, d-Escherichia coli.*	Hydrolyzes phytate

Recently, considerable interest has been shown in the use of phytase as a feed additive, as it not only increases the availability of phosphate in feed but also reduces environmental pollution (Pariza and Cook, 2010). Several other enzyme products are currently being evaluated in the feed industry, including protease to enhance protein digestion, lipases to enhance lipid digestion, β-galactosidases to neutralize certain anti-nutritive factors in non-cereal feedstuffs, and amylase to assist in the digestion of starch in early-weaned animals (Marquardt, 1997).

Phytate and phytate-bound phosphorus (P) is present in all poultry diets and the partial availability of phytate-P has long been recognized. Possibly, Warden and Schaible (1962) were the first to show that exogenous phytase enhances phytate-P utilisation and bone mineralisation in broiler chicks. Nevertheless, three decades elapsed before an *Aspergillus niger*-derived phytase feed enzyme, with the capacity to liberate phytate-bound P and reduce P excretion, was commercially introduced in 1991. It was then considered that the use of microbial phytases would be confined to areas where financial penalties are imposed on excessive P levels in effluent from intensive pig and poultry units (Chesson, 1993). Contrary to this forecast, the inclusion of phytase feed enzymes in monogastric diets has been far more widely accepted and now exceeds that of NSP degrading enzymes (Bedford, 2003). Phytase feed enzymes have more general application as their substrate is invariably present in pig and poultry diets and their dietary inclusion economically generates bioavailable P and reduces the P load on the environment.

The principle rationale for the use of enzyme technology is to improve the nutritive value of feed stuffs. All animals use enzymes in the digestion of food, those produced either by the animal itself or by the microbes present in the digestive tract. However, the digestive process is nowhere near 100% efficient. For example, swine are unable to digest 15-25% of the food they eat. Therefore, the supplementation of the animal feed with suitable enzymes to increase the efficiency of digestion can be seen as an extension of the animal's own digestion process (Pariza and Cook, 2010). In many animal production systems, feed is the biggest single cost and profitability depends on the relative cost and nutritive value of the feeds available. Often, the limiting factor when formulating rations is the animal's ability to digest different constituent parts of the feed raw material differently, particularly the fibre. Despite recent advances, the potential nutritional value of feedstuffs is not achieved at the animal level. This inefficiency in the utilization of nutrients can result in an extra cost to the farmer, the food company and the environment (Sheppy, 2003). Thus, the feeds need to be supplemented with the exogenous enzymes and these are the four main reasons for using enzymes in animal feed:

i) To breakdown anti-nutritional factors that are present in many feed ingredients. These substances, many of which are not susceptible to digestion by the animal's endogenous digestive enzymes, can interfere with the normal digestion, causing poor performance and digestive upsets (Sheppy, 2003).

ii) To increase the availability of the starches, proteins, and minerals that are either enclosed within the fibre-rich cell wall and, therefore, not as accessible to the animal's own digestive enzymes, or bound up in a chemical form that the animal is unable to digest (e,g, phosphorus as phytic acid).

iii) To break down specific chemical bonds in raw materials that are not usually broken down by animal's own enzymes, thus releasing more nutrients.

iv) To supplement the enzymes produced by young animals where, because of the immaturity of their own digestive system, endogenous enzyme production may be inadequate (Sheppy, 2003).

In addition to improving diet utilization, enzyme addition can reduce the variability in nutritive value between feedstuffs and improving the accuracy of feed formulations. Experimental trials have shown that ensuring feed consistency in this way can increase the uniformity of groups of animals, thus aiding management and improving profitability. The general health status of animals can also be indirectly influenced, resulting in fewer of non-specific digestive upsets that are frequently provoked by the fibre components in the feed (Sheppy, 2003).

The inclusion of feed enzymes in poultry diets to enhance nutrient utilization and performance by counteracting the negative influence of targeted substrates has become common place within the last two decades. The role of exogenous enzymes capable of degrading non-starch polysaccharides (NSP) in broiler diets based on 'viscous' grains, including wheat and barley has been elucidated by Bedford and Schulze (1988). Thus, enzyme supplementation in the feed plays an important role in increasing the availability of nutrients and retarding the adverse effect of anti-nutritional factors present in the feed components.

Improvement obtained in the poultry with application of different enzyme preparations

The improvements obtained by adding enzymes to the diet of poultry depends on many factors, including the type and amount of cereal in the diet; the level of anti-nutritive factor in the cereal, which can vary within a given cereal; the spectrum and concentration of enzymes used; the type of bird and their age (young bird tend to respond better to enzymes than older birds). Enzyme supplementation to field bean diets has been shown to be effective in improving chick performance (Castanon and Marquardt, 1989). Addition of a cell-wall degrading multi-enzymes complex and α-galactosidase improved the feed-to-gain ratio of raw high-tannin pea diets fed to chickens, whereas no improvement of chick performance was observed for animals fed with a tannin-free pea variety (Brenes et al., 1993b). Pectinase supplementation of pea-based diets for broiler chickens improved weight gain and feed consumption, whereas feed conversion ratios were unaffected (Igbasan and Guenter, 1996). Pectinase or α-galactosidase supplementation in the diets of chicken increased the performance of the birds. Supplementation with α-galactosidase to the milled diets gave the highest cumulative feed intake, whereas no effect was seen for the crushed diets with larger particle size (Daveby et al., 1998). Pectinase supplementation significantly increased cumulative feed intake on the 10th day for both the milled and the crushed diets, compared to the unsupplemented diets (Daveby et al., 1998). Marquardt et al. (1994) reported an improvement obtained in growing chicks when five different cereals were supplemented with an enzyme preparation high in xylanase and β-glucanase activities. In general, they found a close relationship between the amount of soluble arabinoxylans or β-glucans in the different cereals, their corresponding viscosities, and the performance of the chicks. Brenes et al. (1993a) found that naked oats resulted in the greatest degree of response to enzyme treatment. Enzyme treatment improved weight gain by 46%, apparent metabolizable energy (AME) by 33% and fat digestibility by 193%. On the other hand, adding enzymes to a corn-based diet had no effect on chick performance (Brenes et al., 1993a). Enzyme supplementation also reduces water intake and water content of the excreta, which is probably related to a reduced ability of the partially hydrolyzed water-soluble non-starch polysaccharide (WSNSPS) to absorb water (Marquardt et al., 1994). The excreta with reduced water content is of immense importance, as high moisture excreta will lead to vent pasting; soiled birds and eggs; enhanced ammonia production due to continued fermentation by microorganisms and an increased growth of fungal spores, including those that may be responsible for damage to farmer's lungs (aspergillosis). A number of research studies with layers have reported positive responses to the addition of Avizyme with respect to reduced diet cost (Cook et al., 2000); improved FCR; liveability (Cook et al., 2000); and egg weight. In broilers and ducks, improvements in FCR, body weight, uniformity and nutrient digestibility were reported. Greenwood et al. (2002) reported that addition of Avizyme 1502 into reduced ME diet (2, 3 and 4% in starter, grower and finisher, respectively) improved performance in terms of 42-day body weight and FCR. Additionally, Kidd et al. (2001) found that Avizyme 1500 improved significantly 49-day mortality corrected feed conversion when applied into a diet mimicking industry standard (IS) and a diet with increased lysine and TSAA levels compared to the ones found in IS. These observations indicate that enzyme supplementation of cereal-based diets can have a dramatic effect on the performance and probably also the physiological responses of chickens.

Increased nutrient digestibility and decreased excreta output by supplementing enzymes in the diets of the poultry

In recent years, enzymes have been widely used in monogastric diets to increase nutrient digestibilities and to decrease nutrient waste in the excreta. In densely populated parts of the world, such as Asia and Europe, excretion of large amounts of organic matter, especially those containing high levels of nitrogen and phosphorus, presents serious environmental threats. The large amount of excreta output in the poultry industry is a serious concern for the environment as well as for it results in slower growth performances. Dry-matter digestibility (DMD) in animals ranges from 50 to 80%; the remainder of the dry matter (DM) is lost via the excreta. In the poultry industry, the amount lost via excreta is about 9 000–22 000 t of high-N manure per million birds annually. The effect of enzyme supplementation on DMD in poultry depends on the type of diet and the type of animal. An increase in DMD ranges from 0.9 to 17% in poultry has been reported by enzyme supplementation (Annison and Choct, 1993; Schutte et al., 1995). The enzyme supplementation improved DMD by 17%, apparent metablizable energy (AME) by 24%, and feed-conversion rate (FCR) by 31% (Annison and Choct, 1993).

The enzymes currently used in monogastric diets are predominantly glycanases, which are known to cleave non-starch polysaccharides (NSPs) into simpler form, thereby eliminating their ability to form viscous digesta and improving nutrient digestibilities. The effects of glycanases are generally non-specific, except for their effect on fat, which is known to have a greater effect on saturated fat than on unsaturated fat. Another enzyme which is being used in feed on a large scale is phytase. Phytase is known to increases the utilization of phytate phosphorus. The ability of phytase to enhance the digestion of phytate phosphorus and subsequently to reduce the output of organic phosphorus to the environment has attracted a great deal of interest. In poultry, use of phytase was reported to reduce phosphorus excretion by as much as 40% for broilers (Simons and Versteegh, 1991). When phytase was added to layer diets, increased egg production and positive effects on egg weight and tibia ash were also noted (Simons and Versteegh, 1991).

Cereal by-products, such as rice bran, are important feed ingredients used in Asia, but their efficient use in monogastric diets is hindered by the presence of high levels of NSPs and phytase. Martin (1995) demonstrated that supplementing duck diets with microbial phytase which allowed rice bran to be used at high levels (up to 60%) without detrimental effects. Phosphorus excretion was reduced by 9.6%, and significant decreases in excretion of manganese, copper, and zinc were also noted. Thus, enzyme supplementation in the diets of the poultry increased the nutrient digestibility and decreased the excreta output.

Impact of exogenous enzyme supplementation on growth performance of poultry

The role of exogenous enzyme capable of degrading NSP in broiler diets based on 'viscous' grains, including wheat and barley has been studied by Bedford and Schulze (1998). Supplementation of the maize/soy-based diets with both glucanase and xylanase displayed improvement in feed conversion ratio (FCR) and ileal nutrient digestibility (Cowieson et al., 2010). When both enzymes were added simultaneously, a synergistic effect, resulted in greater benefits than either enzyme independently but less than the sum of the individual effects was observed. The best performance was achieved with the combination of xylanase (16 000 BXU/kg) and glucanase (30 000 BU/kg) (Cowieson et al., 2010). The enzyme (Yemzim B) containing 300 IU of xylanase/g, 20 IU of β-glucanase/g, 20 IU of hemicellulase/g and 260 IU of amylase/g with or without essential oil was incorporated in wheat—soybean meal based, in mash form diet for broilers. Birds fed on diets containing enzyme, essential oil (250 mg/kg) and enzymes plus essential oil (250 mg/kg) had significantly higher weight gain than those given control diet from d 0 to 7 (Basmaciog et al., 2010). Supplementation with enzyme significantly decreased viscosity and increased dry matter of digesta, but did not alter pH of digesta. Enzymes and essential oil had different modes of actions. The supplementation of enzyme with essential oil in diets is more likely to be effective in view of performance, nutrient digestibility, enzyme activities and immune system (Basmaciog et al., 2010).

In another study, Maize-soyabean based diet supplemented with mixture of xylanase, amylase, and protease (XAP) at 650, 1650 and 4000U, respectively, or *Escherichia coli*-derived phytase (1000 phytase units/kg) individually or in combination was fed to the chicks for 21 days (Tiwari et al., 2010). It was found that a cocktail of XAP alone did not improve performance, but phytase supplementation improved weight gain (Tiwari et al., 2010). The enzymes were additive in their effects on growth performance of the chicks. The enzymes had no effect on ileal digestible energy. Ileal N digestibility was higher in diets with XAP or phytase individually compared with negative control diet. Both phytase and XAP individually and in combination improved ileal P digestibility compared with negative control (Tiwari et al., 2010). This study shows that a combination of XAP and phytase improved performance, but the enhancement in performance appears to be due mainly to phytase. Both XAP and phytase were effective in improving P digestibility and retention of chicks receiving nutritionally marginal maize—soybean meal (Tiwari et al., 2010). In the study of Simon et al. (2009), phytase addition (1500 FTU kg^{-1}) to diets containing 4.5 g kg^{-1} total P increased weight gain (733 g *versus* 338 g) and feed efficiency (1.50 *versus* 1.85) of broilers from 0 to 24 days of age. Subsequently, Cabahug et al. (1999) reported that phytase addition (400 and 800 FTU kg^{-1}) to 2.3 g kg^{-1} non-phytate-P diets increased weight gain (18.8%), feed intake (9.0%) and feed efficiency (7.9%) of broiler chicks from 7 to 25 days of age. However, responses to phytase by broilers offered at 4.5 g kg^{-1} non-phytate-P diets were more modest (respective increases of 5.0, 5.0 and 0%), with a significant interaction between non-phytate-P level and phytase addition for weight gain. In general, the responses to phytase in feed intake and weight gain are more robust and consistent than feed efficiency responses. Rosen (2003) reported that feed efficiency

responses to phytase have been declining with time which was attributes to concurrent improvements in broiler strains, feeds and management techniques. Phytase supplementation of P-adequate broiler diets has been shown to generate misleading responses regarding the growth performance, which may be mediated by dietary nutrient specifications (Selle et al., 1999). The magnitude of responses to phytase will be more pronounced with increasing inclusion rates of the feed enzymes and, presumably, greater degradation of phytate. Shirley and Edwards (2003) investigated phytase supplementation of maize–soy broiler diets (4.60 g total P kg^{-1}; 2.72 g phytate-P kg^{-1}); responses in selected parameters to graded phytase inclusion levels to a maximum of 12,000 FTU kg^{-1}. Increasing the phytase inclusion is associated with substantial increases in total tract phytate degradation ranging from 0.403 to 0.948. Moreover, highest phytase inclusion rate of 12,000 FTU kg^{-1} helped in phytate degradation which was correlated with large increases in P retention, tibia ash, weight gain, feed intake, nitrogen (N) retention, feed efficiency, AME and Ca retention (ranked in descending order of significance). At these extreme inclusion rates, there is a possibility that any minor enzymic side-activities that may be present in the phytase preparation may become significant, and thus having an independent impact on nutrient utilization. Increasing phytase from 750 to 1000 FTU kg^{-1} slightly benefited amino acid digestibility; in contrast, weight gain, feed efficiency and AME responses to phytase reached a plateau at 750 FTU kg^{-1}, which is quite different to the observations reported by Shirley and Edwards (2003). Different studies have reported effect of enzymes on nutrient utilization and growth performance of poultry differently. Thus, the inclusion of different feed enzymes in monogastric diets has been far more widely accepted and have been found to be effective (Bedford, 2003).

Effect of exogenous enzyme supplementation on the overall performance of the poultry

Many enzyme supplementations are known to have a positive impact on the overall performance of the poultry. Different feed ingredients are known to shown different level of response to different enzyme supplementation. For example, cereal grains which are not well digested by the poultry are more greatly influenced by enzyme addition than those which are well digested (Lobo, 2000). Some enzyme are known to destroys anti-nutritional factors or increases the digestibility of indigestible nutrients which can help in saving energy and improves amino acid digestibility (Lobo, 2000). In addition, enzymes can be useful to reduce the digesta viscosity which is important for improving fat digestion and for increasing the availability of fat-soluble vitamins. Some enzymes plays a role in improving the efficiency of absorption of natural dietary pigments – chlorophylls and xanthophylls – that are essential for good yolk color in eggs and broiler beaks as well as feet (Nahm, 2007).

The addition of a complex enzyme to broiler diets (Allzyme Vegro, Alltech, Inc., UK) increased true digestibility of all amino acids except valine (Charlton and Pugh, 1995), where substantial increases in the bioavailability of many amino acids for broilers including most limiting amino acids (lysine, methionine, cystine and threonine) were reported. It is well established that the young chick responds to high amino acid density (AAD) diets optimizing growth performance and meat yield (Corzo et al., 2005; Dozier III et al., 2006), but high AAD diets may not be appropriate as broilers approach market weight. Therefore, decreasing dietary AAD late in development should translate to decreased N excretion and ammonia production of the excreta without negatively influencing production efficiency.

Table 2 shows the effect of enzyme supplementation on performance of different type of poultry. Enzyme supplementation is most commonly done by direct addition of an enzyme or enzyme combination to the feed. The most effective enzyme addition to feed has been when the enzyme action was directed at a specific feed component (Table 2). Data from this table indicates that different species require different levels of dietary enzyme supplementation and different basal diets also require different levels of enzyme supplementation, in order to achieve better performance of poultry. Jo' Zefiak et al. (2010) reported that the cereal type as well as the exogenous enzyme supplementation influence the microbiota in broiler chicken caeca, and may have the effect of reducing potentially pathogenic Enterobacteriaceae populations. Influence of supplementation of xylanase to rye-based diets and β-glucanase to barley-based diets is not only limited to the ileal phase. β-glucanase supplementation could have an important impact on gut health in chickens, in particular with respect to the caecal lumen reflux and possible migration of some of the bacterial species to upper parts of the gastro-intestine (Jo' Zefiak et al., 2010).

Table 2. Enzyme Supplementation and their effects on different types of poultry performance.

Source	Type of poultry	Type of enzyme	Amount of enzyme	Increased performance	Based diets
(Mandal et al., 2005)	Pullets, Guinea fowl, Quail	Commercial enzyme preparations	Two levels added (0 or 0.57g/kg feed)	The apparent metabolizable energy (AMEN) value of rapeseed meal did not improve, while sunflower seed meal increased	Solvent-extracted rapeseed and sunflower seed meal
(Wu et al., 2005)	Layers	β-Mannanase	Recommended levels	1. No significant differences in overall average egg production and egg mass among treatments 2. Increased average egg production and egg mass during 5–8 weeks 3. No significant differences in feed intake, production performance and body weight	Corn–soybean meal
(Lazaro et al., 2003)	Layers	Fungal β -glucanase/ xylanase	Four enzyme concen-trations (0, 250, 1250, 2500mg/kg feed)	No effect on egg production of feed efficiency	Wheat, barley and rye-based diets
(Mathlouthi et al., 2003a)	Turkeys	Xylanase β -glucanase	560 and 2800IU/kg feed of xylanase and _-glucanase, respectively	Improved body weight gain and feed efficiency	Wheat or wheat and barley-based diets
Mathlouthi et al., 2003b)	Layers	Xylanase and β -glucanase, used as two enzyme preparations	1. Wheat/barley based diet with 20mg 2. Corn/soybean meal with 20mg	1. Decreased viscosity of wheat, barley, corn and soybean meal. 2. Other beneficial effects were not significant.	1. Wheat/barley based diets 2. Corn/soy-bean meal
(Odetallah et al., 2002a)	Turkeys	Enzyme mix supplementing β -glucanase units (BGU) and endo-xylanase units	BGU: 300g/ton feed, EXU: 300g/ton feed, EXU: 100g/ton feed, BGU 150g/ton feed	Marginal improvement in growth performance depending upon enzyme formulation	Wheat-based diets
(Odetallah et al., 2002b)	Turkeys	Mannan-endo-1,4-_-mannosidase	With or without 100 million units (MU) Hemicell/ton (1MUD106 enzyme activity U)	Some of the adverse effects of anti-nutritional factors of SBM on turkey growth performance can be alleviated by enzyme supplementation	SBM-44 or SBM-48 was found in the basal diet
(Zhang et al., 2000)	Pullets	Five preparations using: *T. viride, T. ressei,* *A niger (Finizym),* *A. niger (SP 249),* *Bacillus subtillis*	1. 0.003125% 2. 0.00625% 3. 0.0125% were added respectively	1. Can accurately evaluate profitability 2. Selects the most profitable cereal	Barley-based diets
(Zhang et al., 2001)	1-d-old single combed white leghorn cockerels	Xylanase	Six amounts of two enzymes: 1. 0, 0.25, 0.75, 2.25, 6.75, and 20.25g/kg feed 2. 0, 0.1, 0.3, 0.9, 0.27, 8.1g/kg feed	1. Evaluate the efficacy of enzyme preparations 2. Predict chick erformance	Rye-based diets

Effect of microbial phytase supplementation on ileal digestibility of amino acids and in reducing the phosphorus excretion into the environment

Many research studies have reported improvements to varying extents in the coefficient of apparent ileal digestibility (CAID) of amino acids following phytase addition to practical broiler diets. Several research studies which have reported the effect of phytase on digestibility of amino acids has been determined. In these studies, the inert markers selected include chromic oxide (Kornegay et al., 1999; Zhang et al., 1999; Camden et al., 2001; Dilger et al., 2004; Onyango et al., 2005), acid insoluble ash (Selle et al., 1999) and titanium oxide (Rutherfurd et al., 2002; Ravindran et al., 2006). It was reported that effect is greater when acid insoluble ash or titanium oxide were used as dietary markers in comparison to chromic oxide. It can be seen that, added phytase increased the mean digestibility coefficient of essential amino acids by 4.4% in the acid insoluble ash and titanium oxide assays, whereas in the chromic oxide assays, the mean digestibility coefficient was increased by 1.4%.

The incorporation of microbial phytases in pig and poultry diets has achieved a good momentum irrespective of the cereal grain base. As reviewed by Selle and Ravindran (2007), the implications of phytate in poultry diets are numerous but essentially, phytases liberate phytate-bound phosphorus (P) and helps in reducing P excretion, which is of ecological importance. Consequently, the combined addition of xylanase and phytase in wheat-based broiler diets is gaining importance. Previous studies have already proved the efficacy of using a combination of enzymes (Ravindran et al., 1999a; Zyla et al., 1999a,b). Xylanase pre-treatment was found to reduce the dietary concentration of arabinoxylan by 15—30% (Denstadli et al., 2010). Xylanase top-dressing tended to improve ileal protein digestibility but, in general, xylanase treatment had no major effect on overall performance in male broilers (Denstadli et al., 2010). Inclusions of xylanase and phytase in broiler diets showed that xylanase increased mean apparent ileal digestibility (AID) coefficients of 17 amino acids by 8.6%, which exceeded the increases of 4.8% and 5.5% obtained by xylanase and phytase, respectively (Selle et al., 2009). Increases in AID were significant (P<0.05) for the majority of amino acids in response to enzyme inclusions with indications of synergistic responses for five amino acids. Xylanase plus phytase increased (P<0.05) nitrogen-corrected AME by 0.63 MJ kg−1, ileal energy digestibility by 0.89 MJ kg−1 and energy AID coefficient by 6.7% relative to the negative control diet (diet supplemented with xylanase: 2000XUkg^{-1}, or phytase: 500 FTU kg^{-1}, or xylanase plus phytase: 2000XUkg^{-1} and 500 FTU kg^{-1}). The enzyme combination increased (P<0.05) coefficients of ileal nitrogen digestibility by 9.1% and 8.0% relative to positive and negative control diets, respectively (Selle et al., 2009). Phytase increased (P<0.05) ileal calcium digestibility by 32.2% and ileal P digestibility by 28.0% relative to the negative control. Ravindran et al. (1999a) reported that xylanase plus phytase increased AID coefficients of 14 amino acids by an average of 8.7% (0.868 *versus* 0.800) in wheat–casein diets. Individually, xylanase increased AID coefficients by an average of 5.0% and phytase by an average of 5.1%. Subsequently, Selle et al. (2003) found that the combination increased AID coefficients of 16 amino acids by an average of 4.6% (0.816 *versus* 0.781) in practical diets based on a wheat–sorghum blend. Phytase increased amino acid AID coefficients by an average of 3.6% but xylanase had a negligible impact (0.4%). The possible mechanisms whereby phytate and phytase influence ileal amino acid digestibility have been considered (Selle et al., 2000, 2006; Cowieson et al., 2006; Selle and Ravindran, 2007). It has also been established that phytate improves the endogenous amino acid flows in broilers which is counteracted by phytase (Cowieson and Ravindran, 2007; Cowieson et al., 2008). These workers reported that sodium phytate addition to an enzyme-hydrolysed casein-based diet markedly increased endogenous amino acid flows with the largest increases being recorded for serine, threonine, glutamic acid, glycine and aspartic acid. The simultaneous inclusion of xylanase and phytase in wheat-based broiler diets generates greater responses than either individual component of the enzyme combination. Inclusion of both xylanase and phytase increase digestibility of amino acids by ameliorating the negative influences on protein digestibility of their respective substrates. The simultaneous inclusion of xylanase plus phytase in wheat-based broiler diets was beneficial in terms of nutrient utilization and growth performance.

Phytase has been shown to increase the CAID (Ravindran et al., 1999b) and true ileal digestibility (CTID) (Rutherfurd et al., 2002) of amino acids of individual feed ingredients in broilers to quite marked extents. Phytase increased the CAID of the

diets given to broilers (Olukosi et al., 2010). Phytase improved the coefficient of the apparent total tract DM retention independently of maize Distillers' Dried Grain with Solubles (mDDGS) and tended to improve the coefficient of apparent phosphorus retention in the diets without mDDGS.

Phytase feed enzymes have more general application as their substrate is invariably present in pig and poultry diets and their dietary inclusion economically generates bioavailable P and reduces the P load on the environment. The incorporation of microbial phytases in poultry diets was induced by the need to reduce P excretion and its loss into the environment, where P pollution is known to be hazardous to water quality. Excessive P drain off into the rivers, lakes and reservoirs are the main reason for their eutrophication (Correll, 1999). Surface runoff from soils with accumulated P accelerates eutrophication, which may result in toxic algal blooms and fish kills (Sharpley, 1999). Consequently any reduction in P excreted by poultry is of benefit to both the environment and sustainable production. Simons et al. (1990) demonstrated the efficient use of microbial phytase to reduce P excretion. They found that 1500 FTU kg^{-1} phytase activity, coupled with reductions in dietary P (7.5–4.5 g kg^{-1}) and Ca (9.0–6.0 g kg−1), reduced P excretion by an average of 61% in two broiler experiments. Zyla et al. (2001) totally eliminated dicalcium phosphate from wheat–soy broiler diets, which reduced non-phytate-P (4.1–1.7 g kg^{-1}) and Ca (9.8–5.9 g kg^{-1}) levels. A combination of phytase and acid phosphatase was included in this modified diet. In a 43-day feeding study, this regime generated a 45% reduction of P in litter (14.8 g/bird *versus* 26.8 g/bird). Additionally, exogenous enzymes significantly enhanced toe ash (164 g kg^{-1} *versus* 150 g kg^{-1}), carcass yield (71.3% *versus* 69.1%) and feed efficiency (1.86 *versus* 1.97), although there was a numerical reduction in weight gain (2124 g/bird *versus* 2215 g/bird). In another study, Paik (2003) reduced non-phytate-P levels in starter (4.5–3.5 g kg^{-1}) and finisher broiler diets (3.5–2.5 g kg^{-1}) without, and with, the addition of 500 FTU kg^{-1} phytase. Decreasing the level of non-phytate-P was proven to reduce P excretion by 14.8%, but the weight gain was decreased. Phytase inclusion further increased the reduction in P excretion by 29.6% but, in contrast, growth performance was not compromised. The impact of phytase on P excretion was based on total P assessments in the above studies. From a limited number of investigations, the phytate-P proportion of total P excreted by poultry offered non-supplemented diets is probably less than anticipated. This proportion ranged from 0.28 to 0.38 in excreta from maize, wheat, triticale and barley-based broiler diets (Pintar et al., 2005) and, in turkeys, a range from 0.16 to 0.32 has been reported (Toor et al., 2005). McGrath et al. (2005) reported that phytate-P represented 0.57 of total P in the excreta of broilers fed non-supplemented diets and 0.50 in those fed phytase- supplemented diets. These relative levels of phytate-P excretion may be indicative of excessive dietary P levels, undigested inorganic P, endogenous P losses and inherent phytase activity in the gut. There is an ecological concern that phytase supplementation may increase P solubility in excreta and litter as soluble P in run-off is more likely to exacerbate eutrophication. For example, Miles et al. (2003) reported that while phytase supplementation of maize–soy diets reduced total P in broiler litter, levels of soluble P were increased (2.85 g kg^{-1} *versus* 2.17 g kg^{-1}). In contrast, however, Applegate et al. (2003) compared standard maize–soy broiler diet with three different phytase-supplemented diets. Overall, phytase reduced total P in fresh litter by 32.2% (7.55 g kg^{-1} DM *versus* 11.14 g kg−1 DM) and soluble P by 43.1% (1.23 g kg^{-1} *versus* 2.16 g kg^{-1}). It is noteworthy that dietary total P concentrations were lower in the study of Applegate et al. (2003). The ecological benefits of phytase supplemented broiler diets, formulated to reduced non-phytate-P specifications, coupled with minimal increases in litter moisture during storage were emphasised by McGrath et al. (2005).

Conclusion and future directions

Enzyme supplementation in the feed play an important role in increasing the availability of nutrients and retarding the adverse effect of anti-nutritional factors present in the feed components. This is the area of research that requires the most attention in the future. For new feed enzyme, products derived from a safe strain lineage should be used, it is important to ensure a sufficiently high safety margin for their intended use, and that the product complies with appropriate specifications for chemical and microbial contamination.

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Emir. J. Food Agric. 2013. 25 (1): 81-88
doi: 10.9755/ejfa.v25i1.9060
http://www.ejfa.info/

AGRICULTURAL ECONOMICS

Model development for wheat production: Outliers and multicollinearity problem in Cobb-Douglas production function

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Abstract

Despite the important role that production function has played in growth literature, few attempts have been made to change the methodology to estimate it. The Cobb-Douglas functions are among the best known production functions utilized in applied production analysis. This paper describes development of a new model based on Cobb-Douglas production function with the used of robust method and partial least squares path modeling for parameter estimation. The new model attempted to solve two main problems in modeling namely the issue of multicollinearity and outliers. Each issue was handled separately but using the same method of least square for parameter estimations. This paper goes on to provide an overview of the measurements and structural criteria needed for model development and, also to introduce a robust partial least squares-path modeling for the Cobb-Douglas production function (RPLS-PM-CD). The researcher hypothesizes that utilization of the minimum covariance determinant (MCD) provides an estimate by the measurement model and expresses the structural relationships between the latent variables through the partial least squares-path modeling (PLS-PM). The inputs and outputs of the RPLS-PM-CD were based on agricultural wheat production data pertaining to Al-Kufra Agricultural Production Project. This paper is more theoretical and should be seen as a new way to estimate Cobb-Douglas production function.

Key words: Cobb-Douglas production function, Wheat production, The minimum covariance determinant (MCD), Partial least squares-path modeling (PLS-PM), Multicollinearity, Outliers, Ordinary least squares (OLS)

Introduction

Production functions are a basic component of all economics domains. As such, estimation of production functions have a long history in applied economics, starting in the early 1800's. Unfortunately, this history cannot be deemed an unqualified success, as many of the econometric problems that hampered early estimation are still an issue today (Ackerberg et al., 2006). A large number of research papers dealing with Cobb-Douglas production functions published in the area of agricultural economics is a testimony to the important role played by these models, by using ordinary least squares regression methodology (Prajneshu, 2008). But the OLS is not the best method. In economic theory, the multicollinearity arising in least squares estimation of the Cobb-Douglas model is not new. It is a problem that emerged with the model itself, when evaluating the 1928 work of Cobb and Douglas (Cobb and Douglas, 1928). On the other hand, in applied economics and econometrics, it has always been highlighted that even if a small amount of data behaves differently from the vast majority of the observations, classical estimations may be affected, leading to results that are not representative of the population. In other words, the presence of outliers might bias the results. The researchers proposed the development of Cobb-Douglas production function parameter through the partial least squares- Path modeling (RPLS-PM) method to be applied on Libyan Agriculture sector data. It attempted to solve two main problems in the model namely the issues of multicollinearity and outliers. Each of the issues was handled separately using the same method of least square for parameter estimations.

Received 27 September 2011; Revised 17 March 2012; Accepted 27 March 2012; Published Online 24 November 2012

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Multicollinearity Problem in Cobb-Douglas Production Function

Murthy (2002) suggestion for access to the best estimate of the Cobb Douglas is to take dissimilar industries such that capital/labour varies, but would be fitted across a heterogeneous group of industries. Olarinde and Manyong (2008) stated that in the economic theory multicollinearity commonly occurs because of the nature of aggregation of economic data (Wethrill, 1986; Gujarati, 2006). They used of ridge regression which overcomes the problem of multicollinearity by adding a small quantity to the diagonal of X^{1}. Zhang and Shang (2009) suggested that the relationships between influential factors and rural infrastructure are often not so complex. Zhang and Shang adopted the PLS method in real data (rural infrastructure) to avoid the preceding limitations of OLS by using Cobb-Douglas production function after log-transformation. In order to verify the precision of the PLS method, they compared this method with the classic methods and found that the PLS methods are obviously more precise than the others.

The minimum covariance determinant (MCD)

The minimum covariance determinant (MCD) method of Rousseeuw (1985) is a highly robust estimator of multivariate location and scatter. Its objective is to find h observations (out of n) whose covariance matrix has the lowest determinant.

Partial least squares- path modeling (PLS-PM)

Partial Least Square- Path Modeling (PLS-PM) is a statistical approach for modeling complex multivariable relationships among observed and latent variables (Vinzi at el., 2009). The structural (or path) coefficients are estimated through ordinary least squares (OLS) multiple/ simple regressions among the estimated latent variable scores. Partial least squares regression (PLSR) can nicely replace OLS regression for estimating path coefficients whenever one or more of the following problems occur: missing latent variable scores, strongly correlated latent variables, a limited number of units as compared to the number of predictors in the most complex structural equation. A PLS-PM is described by two models: (1) a measurement model relating the manifest variables (MVs) to their own latent variables (LVs) and (2) a structural model relating some endogenous LVs to other LVs. The structural model is called the inner model and the measurement model is also called the outer model (Tenenhaus et al., 2005).

Sources of Data and Construction of Variables

The most important projects of agricultural productivity in Libya are in three regions. The first region is the southwest, area of Fezzan, which has four most important agricultural projects, Mknusp Project, Barjuj Project, Aldboat Project, and Deeseh Project. The second region is the area of the central region, Abouchebp which has the Abouchebp project. The third region is the South East region which has the largest agricultural projects in Libya namely, the Alsrero project and the Alkufra project. The latter is one of the most important agricultural project and the largest in Libya. In this paper, data were collected from the Al- Kufra project, involving agricultural production of the wheat crop from 1960 to 2010. This data covered almost all important economic activity inputs. The following variables have been used to estimate the model:

Output Items: the unit for wheat production is tone per hectare.

Input Items: -Land data requirements include: *Harvested area* thousand hectare of land farmed in wheat crop and *water use*: Million cubic meters of water / ha. Agricultural inputs: Average per hectare: *seeds* Ton/ ha, *Chemical fertilizers*: DAP, Urea 46%, TSP, Mixed, Micro elements (Tons / ha), and *Pesticides*: Herbicide, Insecticide, Insecticide (Liter/ ha). Hours of operation hours / day (*Labour* use): Labour input is measured in person - year equivalent of workers directly engaged in production in farming, and *wages*, salaries & admin cost: Average cost per season: Wages & salaries, Social security, Camping costs, Administrative costs. Operating and maintenance: Average cost per season: *Electricity, Fuel, Spare parts, and Oil & lubrications.*

Description of the Model

The production function is specified as a Cobb-Douglas function in the form of:

$$W=\alpha_0\, y_1^{\alpha}\; y_2^{\alpha}\; y_3^{\alpha}\; y_4^{\alpha}\; y_{15}^{\alpha}\; y_6^{\alpha}\; y_7^{\alpha}\; y_8^{\alpha}\; y_9^{\alpha}\; y_{10}^{\alpha}\, y_{11}^{\alpha} \quad (1)$$

where W is the wheat crop output; the coefficient α_0 is the total factor efficiency parameter for composite primary factor inputs in sector i; the parameters $\alpha_1, \alpha_2, \alpha_3, \alpha_4, \alpha_5, \alpha_6, \alpha_7, \alpha_8, \alpha_9$ and α_{10} are production elasticities; y_1 = water; y_2 = land; y_3 = seeds; y_4 = chemical fertilizers; y_5 = pesticides; y_6 = operation hours; y_7 = wages; y_8 = spare parts; y_9 = fuel; y_{10} = oil and lubricants; and y_{11} = electricity. Equation (1) demonstrates that the relationships between the

output and inputs are non-linear. However, by taking the logs of both sides of the equation, the following log-linear relationship is obtained:

$$\log W = \log \alpha_0 + \alpha_1 \log y_1 + \alpha_2 \log y_2 + \alpha_3 \log y_3 + \alpha_4 \log y_4 + \alpha_5 \log y_5 + \alpha_6 \log y_6 + \alpha_7 \log y_7 + \alpha_8 \log y_8 + \alpha_9 \log y_9 + \alpha_{10} \log y_{10} + \alpha_{11} \log y_{11} + e \quad (2)$$

This equation is described as log-linear because both the dependent variable and the regressors have been log-transformed. The coefficients in log-linear equations are elasticities. The Cobb-Douglas production function is estimated using the OLS method. The strength of this paper is embodied chiefly by the development of a Cobb-Douglas production function model based on a robust method and PLS-PM for parameter estimation. The presumption of this investigation is the presence of significant, strong, relationships between the inputs and the wheat production outputs. Besides that, the researcher created four separate data sets, and named groups of data according to variables using the names of the underlying data that represent a particular group.

The researcher denoted; the endogenous latent variable of the *land* and *water* (*LW*) is denoted by η_1. *LW* is formed by two indicator variables y_1, and y_2. The measurement error for *LW* is represented by δ_1 and δ_2. Also, endogenous latent variable for agriculture inputs, *AR* is labeled as η_2. *AR* is formed by three indicator variables $y_3, y_4,$ and y_5 (seed, chemical fertilizer and, pesticides). The measurement error for this indicator variables are represented by $\delta_3, \delta_4,$ and δ_5 respectively. According to Khan and Hossain (2007), wheat output is affected by seed, chemical fertilizer and, pesticides. While; the *Hour operation and Wages (HW*) latent variables consist of exogenous latent variable *HW*, which is symbolized as ξ_1, and two indicator variables x_1 and x_2. and, the measurement error is represented by ε_1 , and ε_2. The *Electricity, Fuel, Spare parts* and, *Oil & lubricants (OP)* model consists of exogenous latent variables and the four indicators, represented by x_3, x_4, x_5 and, x_6. On the other hand, the measurement error is represented by $\varepsilon_3, \varepsilon_4, \varepsilon_5$ *and*, ε_6. The *Output* in the final set deals with relationship between the indicator variables (y_6) and an endogenous latent variables (η_3), and is the model's output. The output model is the form of formative measurement. One way to develop a model of wheat production is based on the concept of inputs and finding correlations between them. Formulation of this model is based on the results of research conducted by Kherallah (2000), Keyzer et al. (2000), Boisvert and Chang (2006), and Khana and Hossaib (2007) confirming a significant correlation between the *Output* and *LW, AR, HW*, and *OP*.

The structure matrix of the measurement model *LW, AR, HW, OP*, and *Output* are respectively as follows:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} \lambda_1 \\ \lambda_2 \end{bmatrix} [\eta_1] + \begin{bmatrix} \delta_1 \\ \delta_2 \end{bmatrix}$$

(3)The measurement model of *LW*

$$\begin{bmatrix} y_3 \\ y_4 \\ y_5 \end{bmatrix} = \begin{bmatrix} \lambda_3 \\ \lambda_4 \\ \lambda_5 \end{bmatrix} [\eta_2] + \begin{bmatrix} \delta_3 \\ \delta_4 \\ \delta_5 \end{bmatrix}$$

(4) The measurement model of *AR*

$$\begin{bmatrix} x_1 \\ x_2 \end{bmatrix} = \begin{bmatrix} \omega_1 \\ \omega_2 \end{bmatrix} [\xi_1] + \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \end{bmatrix}$$

(5)The measurement model of *HW*

$$\begin{bmatrix} x_3 \\ x_4 \\ x_5 \\ x_6 \end{bmatrix} = \begin{bmatrix} \omega_3 \\ \omega_4 \\ \omega_5 \\ \omega_6 \end{bmatrix} [\xi_2] + \begin{bmatrix} \varepsilon_3 \\ \varepsilon_4 \\ \varepsilon_5 \\ \varepsilon_6 \end{bmatrix}$$

(6)The measurement model of *OP*

$$[y_6] = [\lambda_6][\eta_3] + [\delta_6]$$

(7)The measurement model of *Output*

The *Output*, *AR, LW* structural model describes the relationships between the *Output* endogenous latent variables and the *LW, AR, HW*, and *OP* exogenous latent variables. The *Output, AR, and LW* structural model is hence described as:

$\eta_1 = \Lambda_{21}\eta_2 + \Omega_{11}\xi_1 + \Omega_{21}\xi_2 + \zeta_1$ $\eta_2 = \Omega_{12}\xi_1 + \Omega_{22}\xi_2 + \zeta_2$

(8)The measurement model of *LW* (9)The measurement model of *AR*

$\eta_3 = \Lambda_{13}\eta_1 + \Lambda_{23}\eta_2 + \Omega_{13}\xi_1 + \Omega_{23}\xi_2 + \zeta_3$

(10) The measurement model of *Output*

The overall structural and measurement models of *LW, AR, HW, OP*, and the *Output* will be described in Figures 1-9 all as the following:

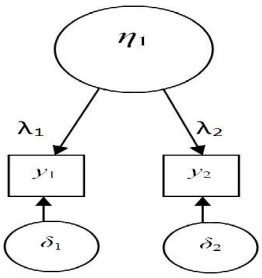

Figure 1. The *LW* Measurement Model

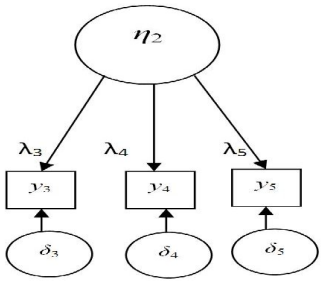

Figure 2. The *AR* Measurement Model

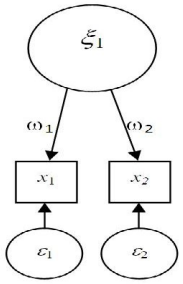

Figure 3.The *HW* Measurement Model

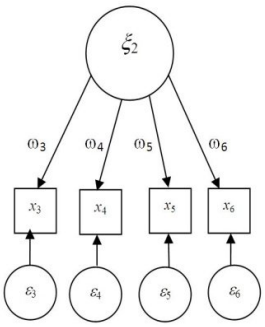

Figure 4. The *OP* Measurement Model

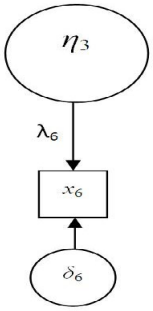

Figure 5. The Measurement Model of *Output*

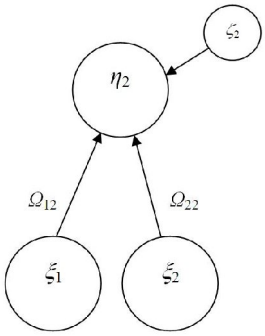

Figure 6. The Structural Model of *AR*

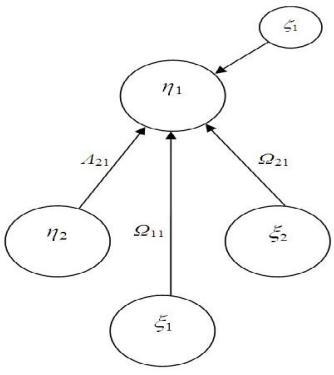

Figure 7. The Structural Model of *LW*

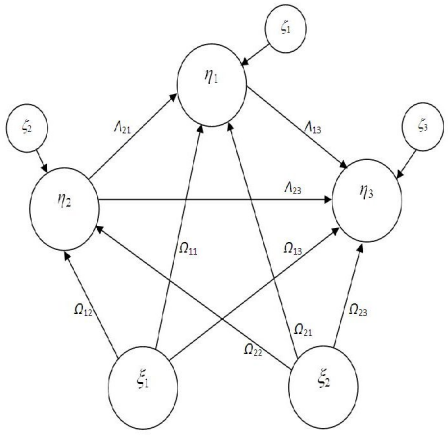

Figure 8. The Structural Model of *Output*

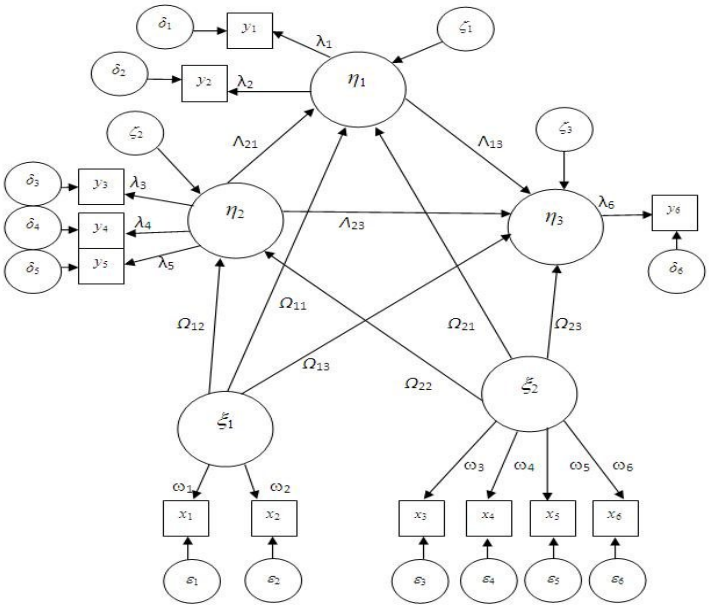

Figure 10. Structural Model and Measurement Model *LW*, *AR*, *HW, OP*, and *Output*.

Results

The Cobb Douglas production function results can be written as follows:

$$\begin{aligned} LogW &= 0.89 - 0.213\ Log\ y_1 + 0.959\ Log\ y_2 - 0.124\ Log\ y_3 \\ &\quad - 0.464\ Log\ y_4 - 0.016\ Log\ y_5 - 0.269\ Logy_6 + 0.259\ Logy_7 \\ &\quad + 1.51\ Logy_8 + 0.001\ Log\ y_9 - .296\ Log\ y_{10} + 0.101\ Log\ y_{11}. \end{aligned} \quad (3)$$

The negative coefficients for the pesticide, water, seeds, hours of operation, and oil and lubrications variables indicate that these variables have an inverse impact on the dependent variable which is in conflict with the logic of production functions.

Outliers and Multicollinearity

In this paper, the distance-distance plot (D-D plot) can be employed for detection of outliers and multicollinearity as was suggested by Field (2000) who considered the correlation coefficients between the independent variables and also the VIF in the diagnosis of multicollinearity. The distance plot showed in Figures 10 below the MCD versus the MD plots which indicate that more than 15 points clearly stand out, whereas the classical analysis shows no points stand out. While, the variance inflation factor (VIF) values are all greater than 10 except for the wages variable. Also, the correlation coefficients results between the independent variables are very high which considered a strong evidence of the existence of multicollinearity. In this paper the researcher seeks to determine a viable technique that can be employed to overcome the outliers and multicollinearity problems in the Cobb-Douglas production function. Therefore, the RPLS-PM is proposed to overcome the process of outliers and multicollinearity in this paper.

Cobb-Douglas Production Function Through Partial Least Squares- Path Modeling (PLS-PM-CD) Model

Evaluation of a research model using PLS-PM analysis consists of two distinct steps. The first step includes assessment of the measurement model and deals with the evaluation of the characteristics of the latent variables and measurement items that represent them. The second step involves the assessment of the structural model and involves evaluation of the relationships between the LVs. And the overall structure model according to the PLS-PM structure provides three different fit indices: the communality index, the redundancy index, and the Goodness of Fit (GOF) index. Results of the assessment demonstrated that the research model successfully passed the test of composite reliability where the Cronbach's Alpha

coefficient was greater than 0.70. Furthermore, the results of the assessment of reliability of the individual measures illustrated that the individual loadings of all items were greater than 0.87, which indicates that the proposed model satisfied the reliability of the individual items as well. On the other hand, the results of the assessment pointed out that the research model successfully passed the test of Average Variance Extracted (AVE) assessment which implies high reliability of the measures. The results presented in Table 1.

Assessment of the structural model: The essential criterion for this assessment is the coefficient of determination (R^2) of the endogenous latent variables and estimates for path coefficients. The results described the relationship between the LVs by correlation matrix. The correlation for the model was greater than 0.70, which implies a fairly strong positive relationship as shown in Table 2. The researcher found that there was a relationship between the LVs which indicates that the developed model performs well. The estimated values for path relationships in the structural model should be evaluated in terms of sign, magnitude, and significance. Assessment of the structural model includes testing for significance of the hypothesized relationships between the research model constructs. Once the path coefficients between the two constructs in the model have been calculated, their significance and the significance level of the path can be evaluated. The results of the bootstrap re-sampling procedure with different numbers of re-samples were very stable with respect to the number of re-samples. The results in the case of 200 re-samples pointed out that the model worked well.

Finally, Assessment of the overall structural model. A global criterion of goodness of fit has been proposed by Tenenhaus et al. (2005), which is the goodness of fit (GoF) index. The GoF index is obtained by finding the geometric mean of the average communality index and the average R^2 value. The value of the GoF index was 0.6882 presented in Table3. This index is bounded between 0 and 1. Since 0.688 is within that range; it is concluded that the structural model works well.

Summary

The body of this work yielded the Cobb-Douglas production function which is used extensively in theoretical and applied research. The design of a Cobb-Douglas production function model consists of few steps. First, the general model structure should be determined; input and output parameters as well as their mutual relationships. Then, parameter values should be determined by first linearizing the models through logarithmic transformation and then applying the method of least squares to the linearized parameters. But the log transformation process does not get rid of all the outliers and multicollinearity problems. At this point the researcher attempted to identify the problems facing this approach of research and to find solutions to identified problems. This research is focused on tackling the problems of outliers and multicollinearity by using MCD and PLS-PM. RPLS-PM-CD model shows that the model consists of five measurement and structure models, and describes the structural relationships between the *LW, AR, HW* and, *OP*, besides their structural relationships with the *Output* based on output and inputs of agricultural wheat production in Al- Kufra Agricultural Production Project, Libya. The path diagram in Figure 9 describes an ideal RPLS-PM-CD model. The terms simple structure and unidimensional measurement are used in the general factor analysis framework to signify a model that meets two conditions: (i) Each latent variable is defined by a subset of indicator variables which are strong indicators of that latent variable, and (ii) each indicator variable is strongly related to the other latent variables. Finally, the findings of the model correspond to very good results and provide important new insights. This supports the point that if it solves problems, not only in agriculture sector but may be the most effective path to macroeconomic development.

Table 1. Reliability assessment of the various interested components.

Construct	Composite Reliability	AVE	Squared Root of AVE	Cronbach's Alpha
LW	0.851186	0.741321	0.861000	0.772577
AR	0.941525	0.843237	0.918279	0.755356
HW	0.932941	0.874349	0.935066	0.858261
OP	0.981022	0.928194	0.963428	0.686936

Table 2. Coefficients of the correlations between the latent variables.

	output	*LW*	*AR*	*HW*
LW	0.888			
AR	0.802	0.925		
HW	0.871	0.845	0.865	
OP	0.751	0.890	0.935	0.914

Table 3. The structural model.

Block	Mult.RSq	AvResVar	AvCommun	AvRedund	Goodness of Fit
Output	0.8148	0.0000	1.0000	0.8148	
LW	0.8632	0.2587	0.7413	0.6399	
AR	0.8797	0.1568	0.8432	0.7417	
HW	0.0000	0.1257	0.8743	0.0000	
OP	0.0000	0.0718	0.9282	0.0000	
Average	0.5115	0.1272	0.8728	0.3600	0.6882
	Inner model		Outer model		

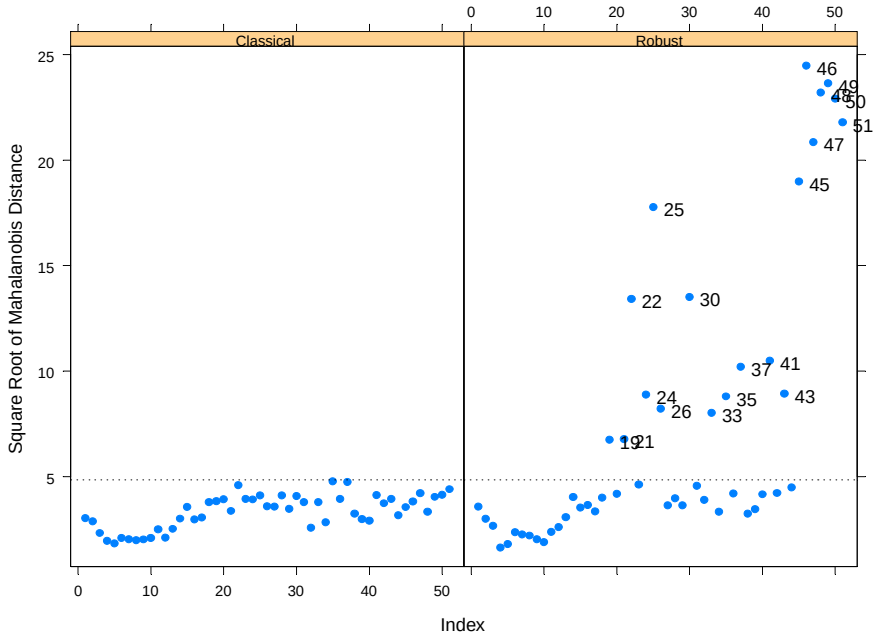

Figure 10. Robust Distances.

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