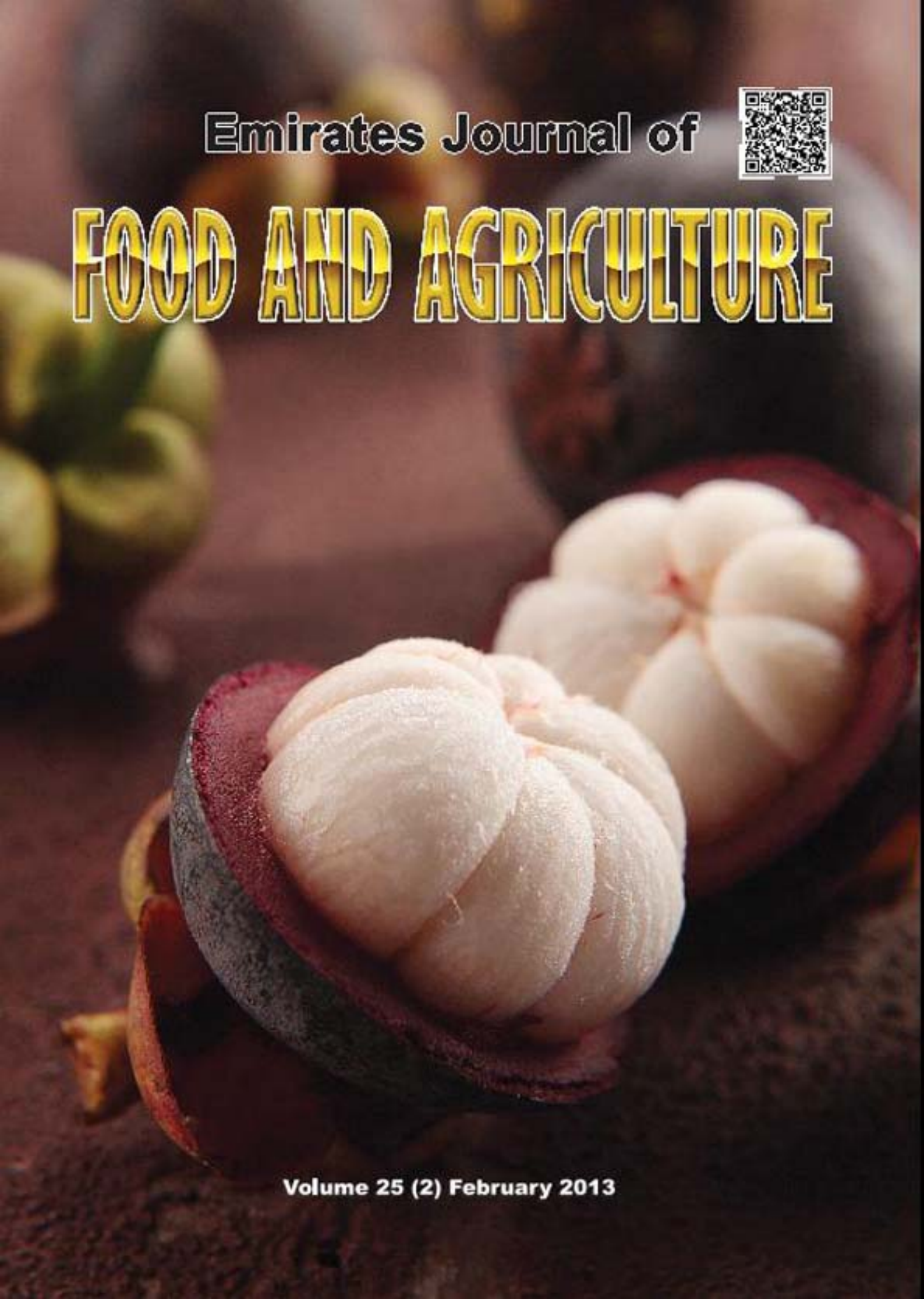


Emirates Journal of FOOD AND AGRICULTURE

Volume 25 (2) February 2013

Emir. J. Food Agric. 2013. 25 (2): 89-96
doi: 10.9755/ejfa.v25i2.11553
http://www.ejfa.info/

NUTRITION AND FOOD SCIENCE

The relation between the texture properties of mangosteen (*Garcinia mangostana* Linn.) and the resonance frequency in detection of the translucent and yellow gummy latex

Rittisak Jaritngam*, C. Limsakul and B. Wongkittiserksa

Department of Electrical Engineering, Faculty of Engineering, Prince of Songkla University, Thailand

Abstract

A nondestructive measurement to predict an internal translucent disorder and yellow gummy latex in mangosteen fruit has proposed by using Vibration Frequency based on Strain gage Sensor (VFSS). This measurement was used vibration with frequency 0 – 50 Hz. The VFSS of 100 mangosteen samples were obtained an evaluation of various existed VFSS signal features based on time and frequency domains. From the experimental results, mass is the best comparing with the other property. This measurement can detect the yellow gummy latex in mangosteen at 35 Hz and 40 Hz.

Key words: Vibration fruit based on Strain gage Sensor (VFSS), Feature extraction, Yellow gummy latex

Introduction

The mangosteen is the queen of fruit and one of the high economical fruit in Thailand. Moreover, it is also useful about an antioxidant for health (Migdalia et al., 2011). However, the problem and quality of mangosteen is measured not only by external factors such as color, shape, size, skin blemishes, latex training and insect damage and must not include the translucent and yellow gummy latex. Currently, by opening the flesh up was the high accuracy for checking the mangosteen. So, the non-destructive inspection is needed. In recent years, the quality determination of mangosteen, Voraphat (1996) studied the effect of water to translucent flesh disorder using a chemical technique. The non-destructive methods of mangosteen in Thailand, a floating technique using differences in specific gravity is currently undertaken for non-destructive detection of translucent flesh disorder in mangosteen. Similarly, Tawatchai et al. (2004) had been proposed the microwave technique to classify the translucent flesh disorder in term of magnitude of the reflection microwave signal.

However, it has been shown that the dielectric of material changes accordingly to its moisture. By the time, the short wavelength infrared spectroscopy used to predict the translucent flesh disorder in intact mangosteen (Sontisuk, 2007). Somchai et al. (1999) had proposed the non-destructive 2D cross-sectional visualization of mangosteen. The X-ray and NMR techniques have been found as new potential tools for non-destructive internal quality evaluation of durian and mangosteen fruits (Yantarasri et al., 1996). The resonance frequency used to detect translucent flesh disorder and yellow gummy latex (Rittisak et al., 2001).

The Physiological disorder for mangosteen is reliable with the water and hollow in the fruit as shown in table 1. In resonance frequency to vibration measurement, Garcio-Romus et al. (2005) had proposed the response of fruit to vibration depends on their mass and shape. Moreover, De Belie et al. (2000) used this methodology to measure pear firmness while the fruits were still on the tree. These authors impacted each fruit near the stem and read the frequency at the opposite side using an accelerometer. Moreover, the non-destructive vibration used to determine the maturity levels of durian (Kongrattanaprasert et al., 2001), the acoustic measurement and an intrusive method for determining tissue firmness were compared to assess the textural properties of kiwifruit (Muramatsu et al., 1997), firmness measurement of muskmelons by acoustic impulse transmission (Junichi et al., 2005), the vibration element analysis to determine firmness evaluation of melon (Nourain

Received 20 March 2012; Revised 04 April 2012; Accepted 06 May 2012; Published Online 28 November 2012

*Corresponding Author

Rittisak Jaritngam
Department of Electrical Engineering, Faculty of Engineer, Prince of Songkla University, Thailand

Email: rittisak.ja@gmail.com

et al., 2005). In addition, the Laser Doppler Vibrometer (LDV) technique was monitor ripening behavior temperature (Shoji et al., 2006), the acoustic impulses were detected internal hollow in watermelon (Diezma et al., 2002), the acoustic response evaluation of the storability of Piel de Sapo melons (Lleó et al., 2005).

In this paper, we propose a non-destructive technique by using VFSS measurement and analysis data by Fast Fourier Transform method (FFT) on frequency domain to detect translucent flesh disorder and yellow gummy latex in mangosteen.

Materials and Methods

Fruit sample and VFSS measurement

Mangosteens were purchased from a local fruit auction in Nakornsri Tummarat, Thailand. The sample was delivered to laboratory to record of signal data on the following by VFSS instrument. About 100 Thai mangosteens were used to study the optimum condition of VFSS measurement and 26 intact mangosteens were used for evaluation the accuracy of translucent flesh disorder detection. Four samples were appeared as translucent flesh disorder with yellow gummy latex while 20 samples were yellow gummy latex. The vibration signal was vibrated in medium sample at frequency of 25 to 40 Hz while amplitude input was 2.5 volt. After, the experiment was completed; the peel of each sample was slit with knife and takes a photograph by digital camera. The samples were cut to record the internal morphology as shown in Figure 1(a), 1(b), 1(c) and 1(d).

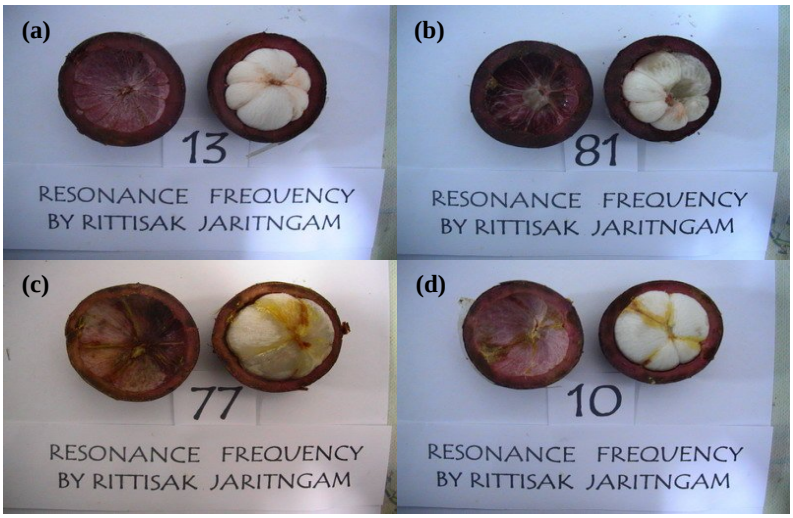
Instruments

The amplitude and frequency of vibration determine have many methods such as ultrasonic and accelerometer and Laser Doppler method can fix sensor with the fruit, there have response of vibration and accuracy. VFSS measurement has used in the vibration measurement of mangosteen.

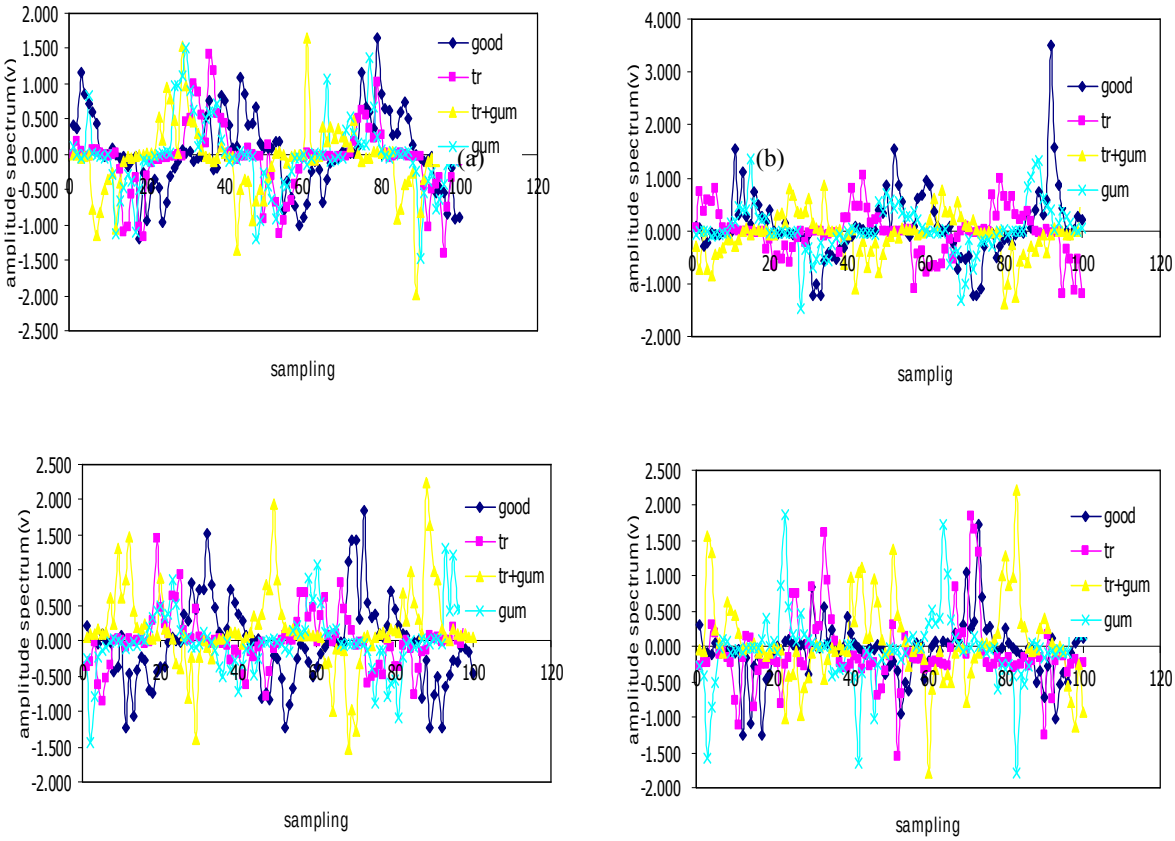
The experimental device consists of the following steps. The first, we put the mangosteen on the base of the prototype, based on vibration set which can vibrate with frequency 0 - 50 Hz. Second, the strain gauge sensor is adjusted by fix on the mangosteen. After that signals from this one sent to amplifier by instrument amplifier circuit (IC = INA 114) to A/D converter. Respectively, we can observe these signals on computer by labview programming and process them by matlab programming for classification of translucent flesh disorder and yellow gummy latex of mangosteen.

Table 1. The different properties of mangosteen (Voraphat, 1996).

No.	Property	Translucent	Yellow Gummy latex	Normal
1	Specific Gravity	Higher than 1	-	Lower than 1
2	Water Volume	Higher than normal	1.21%	
3	Air Volume	Lower than normal	15 time	
4	Density	Higher than normal	3 time	



(a) good sample (b) translucent sample
(c) Translucent and yellow gummy latex (d) yellow gummy latex
Figure. 1 The texture property of mangosteen photograph by digital camera.



(2a) time domain at 25 Hz vibration base, (2b) time domain at 30 Hz vibration base
(2c) time domain at 35 Hz vibration base, (2d) time domain at 40 Hz vibration base
Figure. 2 The differential real time signal of texture property of mangosteen.

Results and Discussion

Data analysis

The resonance frequency in this case, the characteristic of mechanical vibration most parts of the energy radiated from the surface propagates as a transversal wave in the medium when the frequency of low frequency vibration is less than about 1 kHz, the velocity of transversal wave can be expressed as equation (1-4) in medium related to characteristic of medium are given by

$$V_i = \left(\frac{2\left(\mu_1^2 + \omega_b^2 \mu_2^2\right)}{\rho\left(\mu_1 + \sqrt{\mu_1^2 + \omega_b^2 \mu_2^2}\right)} \right)^{1/2} \qquad (1)$$

Where V_i is velocity of vibration. ρ is the density of the medium. ω^b is the angular frequency of vibration. μ_1 and μ_2 are the coefficients of shear elasticity and shear viscosity. Respectively, Velocity of vibration related to frequency and wave propagation of vibration. So, property of shear elasticity can be found by velocity measurement of vibration and wave propagation at low frequency. In equation(1), if the shear elasticity is dominant compared with the shear viscosity so that $\mu_1 >> \omega^b$ then μ_2 is satisfied. The velocity is written as

$$V_i = \left(\mu_1 / \rho\right)^{1/2} \qquad (2)$$

The signal of output vibration fruits, when reference voltage output vibration fruits from the prototype for determine velocity of vibration which can be obtained from

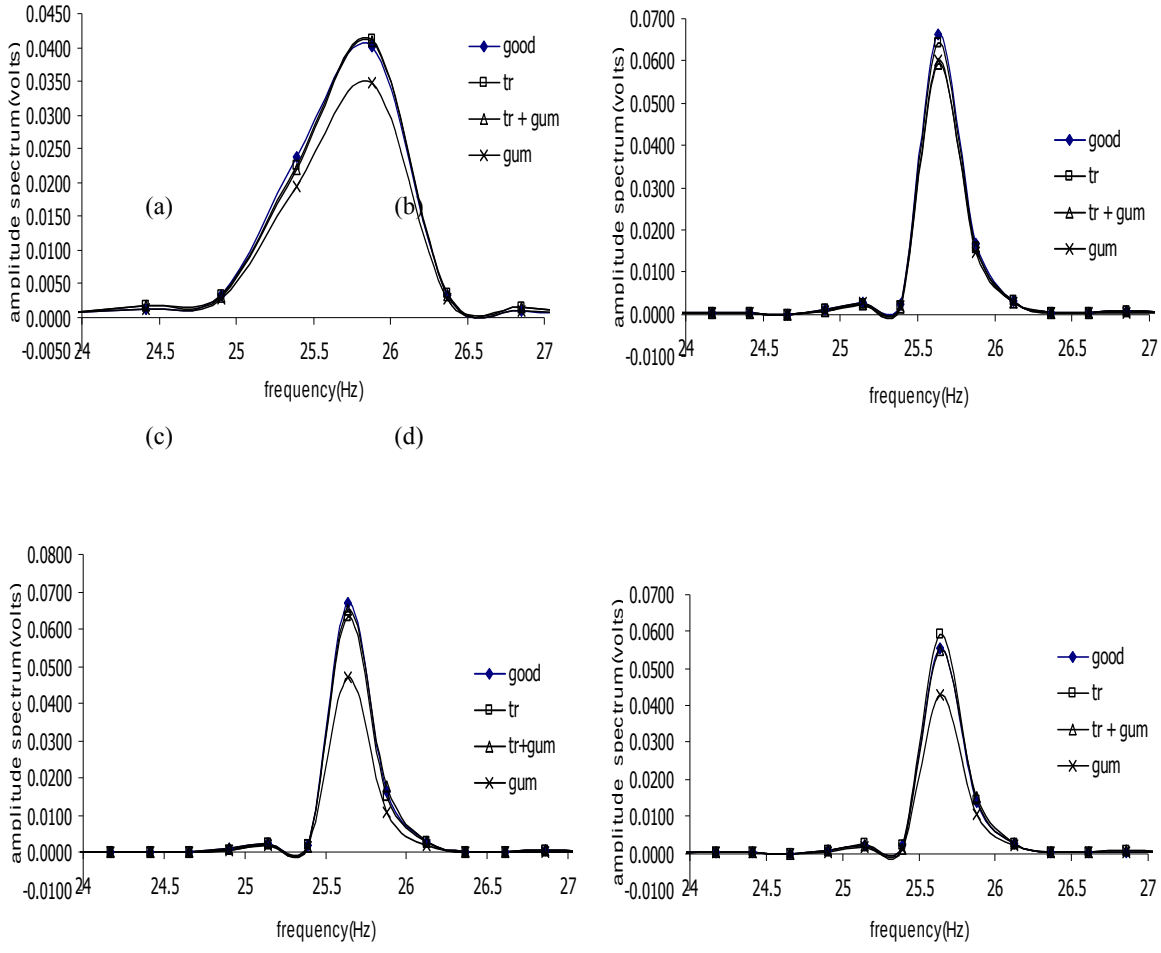
$$V_i = \frac{A}{\alpha T} \qquad (3)$$

$$A = \frac{\alpha V_i}{F} \qquad (4)$$

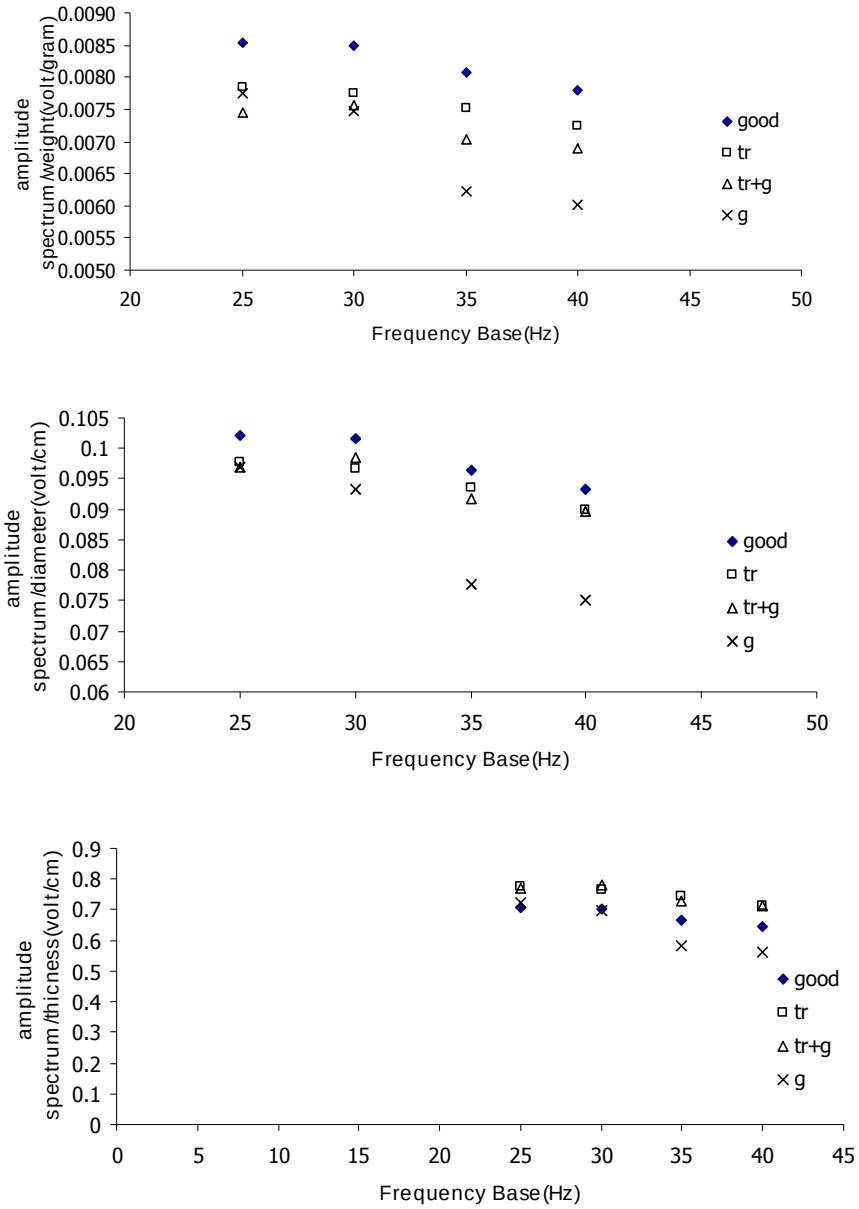
Where A is the voltage of output vibration. T is the time of output vibration. α is voltage of output vibration from the prototype. F is the resonance frequency vibration

The amplitude and frequency of vibration determining have many methods such as ultrasonic and accelerometer and LDV methods which can fix sensor on sample; there have response of vibration accuracy. In this paper, we used VFSS measurement that has been used in the vibration measuring of mangosteen. We choose the frequency vibration between 25 to 40 Hz and input signal is 2.5 Volt, for the mangosteen with different texture property when transfer input signal. Output signal has different amplitudes and frequency has related to internal air cavity, weight and diameter follow to texture property of mangosteen. In Figure 2(a-d), the good mangosteen has patterned signal difference with unusual mangosteen, translucent flesh order and yellow gummy latex.

A Fast Fourier Transform (FFT) is converted from the time signal into a frequency spectrum with frequency 0 to 500 Hz. The resonance frequency, there is usually a high peak, while the spectrum with good mangosteen shown usually one high peak at 26 Hz as we can observe from Figure 3(a-d). From the experiment result, weight and diameter have related with amplitude spectrum. Ratio of amplitude spectrum per weight in every frequency base is the best compared with the other as we can observe from Figure 4(a), there are obtains the ratio is higher than 0.008530 volt/g. There are higher than secondary base vibration about 0.000050 volt/g, the ratio of amplitude spectrum with diameter are the secondary. The lastly, amplitude spectrum per thickness peel are the poor shown in the Figures 4(b) and 4(c). However, this measurement can detect the yellow gummy latex in mangosteen at the 35 and 40 Hz, the amplitude is lowest compared with the other frequency.



(3a) frequency at 25 Hz vibration base, (3b) frequency at 30 Hz vibration base
(3c) frequency at 35 Hz vibration base, (3d) frequency at 40 Hz vibration base
Figure. 3 The typical spectrums for texture property.



(4a) ratio of amplitude spectrum/weight, (4b) ratio of amplitude spectrum/diameter
(4c) ratio of amplitude spectrum/thickness peel.
Figure. 4 The frequency are the best by VFSS measurement comparison
with the other vibration.

Table 2. Mean amplitude spectrum of the texture property in mangosteen.

	Mean amplitude spectrum(Volt)			
Frequency(Hz)	Good	Translucent	Translucent + Yellow gummy latex	Yellow gummy latex
Amplitude				
25	0.519877	0.502694	0.511703	0.494359
30	0.517261	0.496899	0.519601	0.476186
35	0.491520	0.481140	0.483758	0.396590
40	0.475493	0.463501	0.473570	0.382587

Table 3. Mean amplitude spectrum/weight of the texture property in mangosteen.

	Mean amplitude spectrum/weight (Volt/g)			
Frequency(Hz)	Good	Translucent	Translucent + Yellow gummy latex	Yellow gummy latex
Amplitude/weight				
25	0.008530	0.007853	0.007448	0.007761
30	0.008487	0.007762	0.007563	0.007475
35	0.008065	0.007516	0.007042	0.006226
40	0.007802	0.007240	0.006893	0.006006

Table 4. Mean amplitude spectrum/diameter of the texture property in mangosteen.

	Mean amplitude spectrum/diameter (Volt/cm)			
Frequency(Hz)	Good	Translucent	Translucent + Yellow gummy latex	Yellow gummy latex
Amplitude/diameter				
25	0.102097	0.097686	0.097005	0.096933
30	0.101583	0.096557	0.098503	0.093370
35	0.096528	0.093495	0.091708	0.077763
40	0.093380	0.090000	0.089776	0.075017

Table 5. Mean amplitude spectrum/thickness of the texture property in mangosteen.

	Mean amplitude spectrum/thickness (Volt/cm)			
Frequency(Hz)	Good	Translucent	Translucent + Yellow gummy latex	Yellow gummy latex
Amplitude/thickness				
25	0.705875	0.775671	0.769479	0.725665
30	0.702323	0.766728	0.781356	0.698989
35	0.667372	0.742412	0.727456	0.582151
40	0.645612	0.715195	0.712137	0.561596

From the experiment, the Table 2 presents the frequency based vibration within 25 and 30 Hz comparison with the amplitude spectrum. There are the best values about 0.476186 – 0.519877 volt and are higher than the frequency vibration based at 35 and 40 Hz which are valuable about 0.382587 – 0.491520 volt. Moreover, the amplitude spectrums of the good sample are higher than the other sample fruits. Hence, the yellow gummy latex sample can be detected at the frequency based vibration which has the value lowest than the other sample.

In Table 3, it is the value of amplitude spectrum per weight. From the experimental results, there are the same trend with the experiment from Table 2, the ratio of amplitude spectrum per weight in normal sample are higher than the other fruits are about 0.007802 – 0.00853 volt/g on the frequency based vibration. In addition, the frequency based vibration at the 25 Hz has the highest ratio in the 40 Hz frequency based vibration, the ratio has the poor. Moreover, the translucent flesh disorder and translucent flesh disorder combine with the yellow gummy latex are similar to 0.007240 – 0.007853 volt/g and 0.006893 – 0.007448 volt/g. In the part, the mangosteen has yellow gummy latex symptoms only will be the ratio valuable was poor in the same vibration. From the experiment result, the trend of mangosteen can detect in the all frequency based vibration and sorts out mangosteen have yellow gummy latex in 35 and 40 Hz frequency based vibration.

Table 4, represents the value of amplitude spectrum per diameter. From the experimental results, there are the same trend with the experiment from Table 2 and 3, the ratio of amplitude spectrum per diameter in normal sample are higher than the other (about 0.093380 – 0.102097 volt/cm) on the frequency based vibration. In addition, the frequency based vibration at the 25 Hz has the highest and in the 40 Hz frequency based vibration, the ratio has the poor. Moreover, in the value of mangosteen are the translucent flesh disorder and translucent flesh disorder combine with yellow gummy latex are similar to 0.090000 – 0.097686 volt/cm and 0.089776 – 0.097005 volt/cm. Respectively, in the part, the ratio valuable of mangosteen having yellow gummy latex symptoms only was poor in the same vibration. From the experimental results, the trend of mangosteen can detect in the all frequency based vibration and sorts out mangosteen

group have yellow gummy latex in 35 and 40 Hz frequency based vibration.

Table 5 shows the value of amplitude spectrum per thickness peel. From the experiment result, the good mangosteen has ratio value of translucent flesh disorder and translucent flesh disorder combination with the yellow gummy latex between 0.645612 – 0.705875 volt/cm at the frequency based on 25, 30, 35 and 40 Hz. Moreover, the frequency based vibration is 25 Hz has highest and has lowest at the frequency base vibration about 40 Hz. However, the translucent flesh disorder and translucent flesh disorder combination with the yellow gummy will be the ratio valuable lowest which about 0.561596 – 0.725665 volt/cm in the same frequency based vibration.

Conclusion

The VFSS of mangosteen fruit was sufficient to use for translucent flesh disorder and yellow gummy latex detection in mangosteen. The texture property can be classified into 4 groups (good, translucent flesh disorder, translucent flesh disorder combination with yellow gummy latex and yellow gummy latex) vibration based on 25 Hz, where the best amplitude and ratio of amplitude with diameter are the same. Frequency based vibration at 35 and 40 Hz can be classify as yellow gummy latex, the amplitude is lowest than the other frequency based on vibration. However, the accuracy can be increased by rejection of other effects such as hardening pericap, fruit size and skin color before evaluation.

Acknowledgement

The authors acknowledge the support by the graduation scholarship, Prince of Songkla University (PSU) and would like to thank Dr. Kittinan Maliwan, Lecturer in Mechanical Engineering, PSU for help technical in providing vibration set as well as Electrical Engineering (EE) department for their Kind technical help and use of laboratory.

References

De Belie, N., S. Schotte Lammertyn, J. Nicolai, B. J. De Baerdemaeker. 2000. Firmness changes of pear fruit before and after harvest with acoustic impulse response technique. J. Agric. Eng. Res. 77(2):183-91.

Diezma, B., M. Ruiz-Altisent and B. Orihuel. 2002. Acoustic impulse response for detecting hollow heart in seedless watermelon. Acta Hort. 599:249-256.

Garcia-Ramos, F. J. C. Valero, I. Homer, J. Ortiz-Cañavate and M. Ruiz-Altisent. 2005. Non-destructive fruit firmness sensors: a review. Spanish J. Agric. Res. 3(1):61-73.

Junichi, S., M. I. Al-Haq and M. Tsuta. 2005. Application of Portable Acoustic Firmness Tester for Fruits. The fifth International Conference on Information and Technology for Sustainable Fruit and Vegetable Production, Montpellier, France. p.439-443.

Kongrattanaprasert, S., S. Arunrungrusmi, B. Pungsiri, K. Chamnongthai and M. Okuda. 2001. Nondestructive Maturity Determination of Durian by Force Vibration. The IEEE International Symposium on Circuits and System (ISCAS), Sydney, Australia. p.441-444.

Lleó, L., P. Barreiro, A. Fernández, M. Bringas ,B. Diezma and M. Ruiz-Altisent. 2005. Evaluation of the storability of *Piel de Sapo* melons with sensor fusion. The fifth International Conference on Information and Technology for Sustainable Fruit and Vegetable Production, Montpellier, France. p.523-531.

Migdalia, A., A. Bello, L. Rastrelli, M. Montelier, L. Delgado and C. Panfet. 2011. Antioxidant properties of pulp and peel of yellow mangosteen fruits. Emir. J. Food Agric. 23(6):517-524.

Muramatsu, N., N. Sakura, R. Yamamoto, D. J. Nevins, T. Takahara and T. Ogata. 1997. Comparison of non-destructive acoustic method with an intrusive method for firmness measurement of kiwifruit. Postharvest Biol. Technol. 12:221-228.

Nourain, J., Y. Yi-Bin, W. Jian-Ping, R. Xiu-Qin and Y. Chao-Gang. 2005. Firmness evaluation of melon using its vibration characteristic and finite element analysis. J. Zhejiang Univ. B. (6):483-490.

Rittisak, J., C. Limsakul, S. Sdoodee, S. Jaritngam and M. Mani. 2001. To detect gumming fruit of Mangosteen (*Garcinia mangostana* Linn.) by the autoregressive model analysis method. J. Agric. Sci. 32:1-4.

Shoji, T., N. Sakurai, J. Zebrowski, H. Murayamad, R. Yamamotoe and D. J. Nevins. 2006. Laser Doppler vibrometer analysis of changes in elastic properties of ripening 'La France' pears after postharvest storage. Postharvest Biol. Technol. 42:198–207.

Somchai, A., D. Khawaparisuth and K. Chamnongthai. 1999. Nondestructive 2D Cross-Sectional Visualization of A Mangosteen. The fifth International Symposium on Signal Processing and its Applications, Brisbane, Australia. p.443-445.

Sontisuk, T. 2007. Non-destructive prediction of translucent flesh disorder in intact mangosteen by short wavelength near infrared spectroscopy. Postharvest Biol. Technol. 43:202–206.

Tawatchai, T., N. Jittiwaranghl, P. Kumhoml and K. Chamnongthai. 2004. Non-Destructive Grading of Mangosteen by Using Microwave Moisture Sensing. International Symposium on Communications and Information Technologies, Sapporo, Japan. p.650-653.

Voraphat L. 1996. Effect of Water on Physiological Disorders of Mangosteen Fruit (*Garcinia Mangostana Linn*). Developmental Physiology, Master of Science (Agriculture) Department of Horticulture, Kasetsart University, Thailand.

Yantarasri, T., C. Sivasomboon, J. Uthaibutra and J. Sornsrivichai. 1996. X- ray and NMR for nondestructive internal quality evaluation of durian and mangosteen fruits. Acta Hort. 464:97-101.

Emir. J. Food Agric. 2013. 25 (2): 97-101
doi: 10.9755/ejfa.v25i2.11184
http://www.ejfa.info/

NUTRITION AND FOOD SCIENCE

Determination of factors effecting on the hardness of soft peanut tofu using screening model

Quoc Le Pham Tan*, Hieu Ho Thi Thanh, Nhu Duong Thi Ha and Thao Dang Thi Thu

Institute of Biotechnology and Food Technology, Ho Chi Minh City University of Industry, Ho Chi Minh City, Vietnam

Abstract

Soft tofu from soy milk appeared for long time ago. However, soft tofu from peanut milk has not been produced much. There has been little research, especially on factors effecting on the hardness of soft tofu. In this research, investigation on main factors (The heated time, $CaSO_4$ content and the pressing force) affecting the structure of tofu products was conducted at the laboratory scale. The hardness of soft peanut tofu was evaluated using the Screening model. This research also shows the dependence of the heated time, $CaSO_4$ content and the pressing force on the hardness of soft tofu. Those factors were not absolutely interacted together. There is the covariant between the heated time, $CaSO_4$ content and the pressing force with the hardness of soft tofu and all of these factors strongly affected the hardness. The optimal hardness was 0.35 N with the heated time was 4.5 min, $CaSO_4$ content was 2.75 % and the pressing force was 40 N.

Key words: $CaSO_4$, Hardness, Lì peanut, Screening model, Soft tofu, Soy milk

Introduction

Tofu, a Chinese traditional food, has been known as the most nutritious and healthiest food. Tofu was widely used by vegetarian diet with a variety of recipes (Veronica, 2008). It made the easy family meals and there are many reasons to do, for instance: cheap, delicious, nutritious, low calories and it can help to control a few health problems (Afamily, 2011). Peanut tofu was quite known to some Asia countries, while it was almost unknown to western countries. We can process tofu from soybean and peanut by many ways. Peanut milk and soft tofu were produced with slight modifications (Thieu, 1996).

Optimization of conditions for processing is one of the most critical stages in the development of an efficient and economic bioprocess. Statistical methodologies involved used mathematical models for designing fermentation processes and analyzing the process results (Bas et al., 2007). Screening model is a simple mathematical model which can find the important factors as the first phase of a project using linear and interaction models.

Therefore, the objective of the present study was to determine the relationship of some factors which affect to the hardness of soft tofu, contributing to the development of the tofu industry in general.

Materials and Methods

Materials

Lì peanut were obtained from Tay Ninh province in Vietnam. Average weight of 1000 seeds is 420g, diameter 7-8mm. Seeds do not have insects and have characteristic odour.

$CaSO_4.2H_2O$ was obtained from Xilong Chemical Company (China).

Treated water (pH=6.7) was obtained from Green Solution Company (Vietnam).

Muslin cloth was obtained from Thanh Cong Company (Vietnam).

Methods

Soft tofu processing

Peanut grains (80 g) were soaked in 200 mL (1:2.5, w/v) of tap water and stored at 28-30 ^oC for 4.5 hours. The soaked peanut was ground with 800 mL water for 5 min at a high speed in Philips Blender (Volume of tank: 2000 mL, 500 W, 15000 r/m, China). After grinding, the slurry was filtered through a muslin cloth and squeezed by hand to obtain peanut milk (You et al., 2012).

Received 29 February 2012; Revised 01 June 2012; Accepted 16 June 2012; Published Online 28 November 2012

*Corresponding Author

Quoc Le Pham Tan
Institute of Biotechnology and Food Technology, Ho Chi Minh City University of Industry, Ho Chi Minh City, Vietnam

Email: lephamtanquoc@yahoo.com

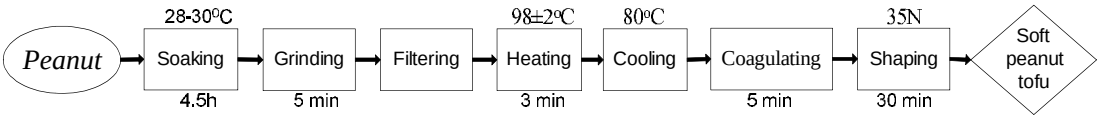


Figure 1. The soft peanut tofu processing.

The peanut milk was heated to boiling point in 98±2°C by the heated machine Midea (1900 W, China), maintained with 3 min (Standard level) while stirring by hand stirrer and cooled to 80°C in the condition room at 30±1°C, relative humidity 60±2%. Soft tofu was prepared by coagulating the peanut milk using the coagulants $CaSO_4$ 2.5% (w/w, standard level). The prepared coagulant was added to the milk and mixture was vigorously stirred for 30 seconds by hand stirrer. The mixture was allowed to stand for 5 min when the curd was separated from the whey and transferred to a wooden mould (6x10 cm) for pressing. The curd was covered by the muslin cloth in the wooden mould. The curd was pressed for 30 min at 35 N (Standard level) letting the water flows out through the bottom hole of the wooden mould. After pressing, tofu was taken out from mould and cut into small samples of 4 x 3 x 2 cm (Quoc et al., 2011). We used INSTRON 5543 mechanical tester (Speed of 5 mm/s, diameter of sensor 3 mm, USA) to measure the hardness of soft tofu.

Figure 2. The soft peanut tofu after taking out the wooden mould.

Experimental design

Screening models were used to determine a few main factors that can affect to the response. In this research, there are three factors including the heated time (x_1), $CaSO_4$ content (x_2) and the pressing force (x_3). It can affect to target the hardness of soft tofu (y). Influence of factors to target function was described according to equation below:

$$y = b_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i<j}^{n} b_{ij} x_i x_j \quad (1)$$

In this study, n-value was 3 so equation (1) can be written:

$y = b_o + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$ (2) (Canh, 2004).

A common experimental design is one with all input factors set at two levels each. These levels are called `high' and `low' or `+1' and `-1', respectively. A design with all possible high/low combinations of all the input factors is called a full factorial model in two levels (Mary et al., 2003). The design contains a total of 11 experimental trials with a full factorial model fashion and the replications of the central points.

Statistical analysis

The hardness of soft tofu was determined by actual response value. The data reported represent its mean. Statistical significance was evaluated using the Analysis of Variance (ANOVA) and $p<0.05$ was considered as significant (Tuan, 2008). Optimum parameters were defined by the software Modde version 5.0.

Results and Discussion

The hardness of tofu was determined according to Table 2. Based on the full factorial model, result of experimental analysis was presented in Table 3 and 4. According to the ANOVA table, the regression model is significant at the considered confidence level (95%) since the regression has $p_{value}=0.042$ (<0.05).

Table 1. Codes and actual levels of the in dependent variables for design of experiment using model (in this case is screening model/full factorial model).

Independent variables	Symbols	Coded levels		
		-1	0	+1
The heated time (minute)	X_1	1.5	3	4.5
$CaSO_4$ content (%, w/w)	X_2	2.25	2.5	2.75
The pressing force (N)	X_3	30	35	40

Table 2. Two level full factorial composite model and experimental responses of dependent variable y (The hardness of soft tofu, N).

Run No	Coded levels			Real values			The hardness of soft tofu (N)	
	x_1	x_2	x_3	X_1	X_2	X_3	Observed	Predicted(*)
1	-1	-1	-1	1.5	2.25	30	0.22	0.22068
2	+1	-1	-1	4.5	2.25	30	0.27	0.27318
3	-1	+1	-1	1.5	2.75	30	0.23	0.23318
4	+1	+1	-1	4.5	2.75	30	0.28	0.28068
5	-1	-1	+1	1.5	2.25	40	0.23	0.23318
6	+1	-1	+1	4.5	2.25	40	0.27	0.27068
7	-1	+1	+1	1.5	2.75	40	0.3	0.30068
8	+1	+1	+1	4.5	2.75	40	0.33	0.33318
9	0	0	0	3	2.5	35	0.25	0.26818
10	0	0	0	3	2.5	35	0.28	0.26818
11	0	0	0	3	2.5	35	0.29	0.26818

(*): Running the software Modde version 5.0 to predict obtained model

Table 3. Analysis of variance (ANOVA) for the full factorial model.

y	DF	SS	MS	F	p	SD
Total	11	0.8023	0.0729364			
Constant	1	0.791136	0.791136			
Total Corrected	10	0.0111638	0.00111638			0.0334122
Regression	6	0.0101751	0.00169586	6.8614	**0.042**	0.0411808
Residual	4	0.000988635	0.000247159			0.0157213
Lack of Fit	2	0.00012197	6.09848e-005	0.140734	0.877	0.00780928
Pure Error	2	0.000866666	0.000433333			0.0208167

Table 4. Results of regression analysis of the full factorial model.

y	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	0.268182	0.00474015	5.84382e-007	0.0131608
x_1	0.02125	0.00555831	0.0187284	0.0154324
x_2	0.01875	0.00555831	0.0279575	0.0154324
x_3	0.01625	0.00555831	0.0430904	0.0154324
x_1*x_2	-0.00125003	0.00555831	**0.833084**	0.0154324
x_1*x_3	-0.00375001	0.00555831	**0.536873**	0.0154324
x_2*x_3	0.01375	0.00555831	**0.0686682**	0.0154324
N = 11	Q^2 =	0.748	Cond. no. =	1.1726
DF = 4	R^2 =	0.911	Y-miss =	0
	R^2_{Adj} =	0.779	RSD =	0.0157

The table shows coefficients in the regression equation. The heated time, $CaSO_4$ content and the pressing force are absolutely independent, do not interact together ($p_{value}>0.05$) and have influence to the hardness of tofu (Table 4). Factors have $p_{value}<0.05$, it means these factors effect on the response. Regression equation as below presents dependence of the hardness of soft tofu on three factors quoted above:

$$y = 0.268 + 0.021x_1 + 0.019x_2 + 0.016x_3 \quad (3)$$

Equation (3) showed the regression coefficients of linear term x_1, x_2 and x_3. The pressing force has less impact on the hardness of tofu than $CaSO_4$ content and the heated time. With first-level coefficients: the heated time, $CaSO_4$ content and the pressing force were covariant when comparing with the obtained hardness of soft tofu (The heated time had the strongest influence level).

Table 4 showed the experimental yields fitted the polynomial equation of the first degree well as indicated by high R^2 (Coefficient of determination) value was 0.911. The R^2_{adj} was 0.779 and the Q^2 was 0.748, which indicates that the model is good. For a good statistical model, the R^2 value should be in the range of 0–1.0, and the nearer to 1.0 the value is, the more fit the model is deemed to be; predictive ability of model manifest by Q^2 with fail-safety achieved R^2.

In Figure 2 and 3, the three-dimensional contour plots and response surface plots are

displayed according to Equation (3). The graph determined the contribution of the heated time, $CaSO_4$ content and the pressing force on the hardness of soft tofu. The response surface in this research was plane rather than a curved surface. The more heated time, $CaSO_4$ content and the pressing force increase, the harder the hardness was in interval research. Heating the peanut milk denatured the protein, the polypeptide chain was extended, ($-COO^-$) group appeared and linked protein chain together. Protein was concentrated and coagulated. Increasing content of calcium sulphate was tightened protein links until no more carboxyl functional group ($-COO^-$) available. The increase of ion Ca^{2+} maybe influences the taste of tofu. The heat will rise to the hydrophobic links, increase hydrophobic links between the polypeptide, thereby increasing the link between proteins by the ($-COO^-$), made the coagulation protein. As pressing force increases, the hardness increases, this is because the water went out from the curd and created good conditions for arranging structure protein chain. The volume of tofu decreased and protein density was higher than previous curd. The tofu becomes the strongest curd (Quoc et al., 2011).

Using the software Modde 5.0 optimized to predicting the result of optimum condition from regression equation which were the heated time 4.5 min, $CaSO_4$ content 2.75 %, the pressing force 40 N and the hardness of soft peanut tofu 0.3332 N

We conducted a re-test with some received parameter. The hardness of soft tofu achieved 0.35 N with an error of 5 % (Compare with predictive model). Correspondingly, this result has a high reliability.

The parity plot shows a satisfactory correlation between the experimental values and predictive values of the hardness of soft tofu (N) using software Modde 5.0 in 11 experiments shown in Table 2. The result shows this was linear correlation (Figure 4). The matching quality of the data obtained by the model proposed in Equation (3) was evaluated considering the correlation coefficient, R^2, between the experimental and modeled data. The mathematical adjustment of those values generated a R^2. The R^2 value should be in the range of 0–1.0, R^2 was near to 1.0 so the model was apparently better. In this case, R^2 = 0.911 and it was the fit model, revealing that the model only could not explain 8.9% of the overall effects, showing that it is a robust statistical model.

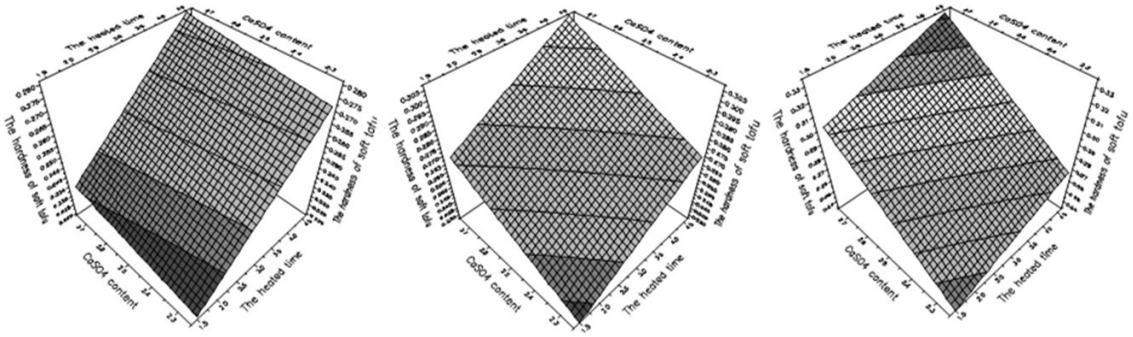


Figure 3. Response surface plot of the hardness of soft tofu when the pressing forces were 30, 35 and 40N.

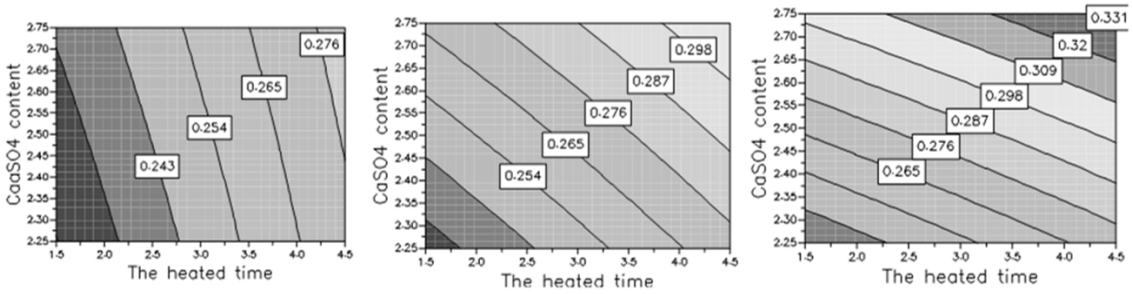


Figure 4. Isoresponse contour plot of the hardness of soft tofu when the pressing forces were 30, 35 and 40N.

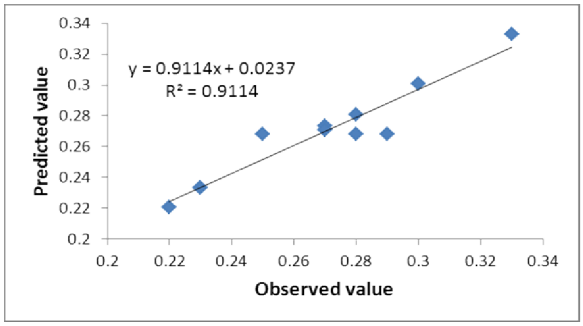


Figure 5. Parity plot showing the distribution of experimental versus predicted values by the mathematical model of the y values.

Conclusions

Conventional optimization studies are time consuming and expensive. To overcome these problems, a screening model was used for the optimization of process conditions. From the present study, it is evident that the use of statistical process condition optimization approach, full factorial model has helped to locate the most significant conditions with minimum effort and time. From studies quoted above, we come to a conclusion that the heated time, CaCO4 content and the pressing force were the main factors that have strong influence on the hardness of soft peanut tofu.

Using the optimal method of target function, we exposed regression equation and this equation can be applied on actual model:

$y = 0.268 + 0.021x_1 + 0.019x_2 + 0.016x_3$

Based on the regression equation, three factors including the heated time, CaSO4 content and the pressing force were determined affect the hardness of soft tofu. These factors were absolutely independence, not interactive together, level of effects did not change much and it had the covariant with hardness. This model had the high R2, R2adj and Q2 and was absolutely according to actual production. It was quite easy to select and modify some parameters in tofu processing.

Acknowledgement

The authors gratefully acknowledge the Institute of Biotechnology and Food Technology, Ho Chi Minh City university of Industry for providing necessary facilities for the successful completion of this research work.

References

Bas, D., H. Ismail and J. Boyaci. 2007. Modeling and optimization. Usability of response surface methodology. J. Food Eng. 78(3):836–845.

Canh Nguyen. 2004. Methods of Optimization. Ho Chi Minh city University of Technology Press (Vietnamese version). Ho Chi Minh City. Vietnam.

http://afamily.vn/suc-khoe/20111129014430271/7-ly-do-de-bo-sung-dau-phu-vao-bua-an-cua-ban/. December 4th, 2011.

Mary, N., C. Croarkin and W. Guthrie. 2003. Engineering Statistics Handbook, Statistical Engineering Division. NIST 2003.

Quoc, L. P. T., Hieu Ho Thi Thanh, Nhu Duong Thi Ha and Thao Dang Thi Thu. 2011. Research soft tofu from peanut milk. World J. Sci. Technol. 1(11):1-7.

Thieu, V. P. 1996. Soybean – Technical cultivating and product processing, Agriculture Press (Vietnamese version). Ho Chi Minh City. Vietnam.

Tuan, V. N. 2008. Analyse data and plot by R. Viet Nam National University-Ho Chi Minh city Press (Vietnamese version). Ho Chi Minh City. Vietnam.

Veronica, A. O. 2008. Effect of different coagulants on yield and quality of tofu from soymilk. Eur. Food Res. Technol. 226(3):467-472.

You, S. O., Joon-Ho Hwang and Sang-Bin Lim. 2012. Physiological activity of tofu fermented with mushroom mycelia. Food Chem. 133(3):728-734.

Emir. J. Food Agric. 2013. 25 (2): 102-108
doi: 10.9755/ejfa.v25i2.7590
http://www.ejfa.info/

PLANT SCIENCE

Isolation and characterization of salt-tolerant rhizobia native to the desert soils of United Arab Emirates

Samrudhi R. Sharma[1], N. Kameswara Rao[2*], Trupti S. Gokhale[1] and Shoaib Ismail[2]

[1]*Birla Institute of Technology and Science (BITS) Pilani – Dubai Campus, Dubai International Academic City, Dubai, United Arab Emirates*
[2]*International Center for Biosaline Agriculture (ICBA), P.O. Box 14660, Dubai, United Arab Emirates*

Abstract

The salinity tolerance of naturally occurring rhizobia, isolated from the root nodules of three leguminous plants, namely sesbania (*Sesbania sesban*), lablab (*Lablab purpureus*) and pigeonpea (*Cajanus cajan*), growing at a research farm in Dubai (United Arab Emirates) was studied. Eight isolates identified from colony morphology and gram staining reaction, when cultured on yeast extract-mannitol agar medium (YEMA) supplemented with different concentration of sodium chloride (NaCl), produced colonies even at salinities as high as 40 dS m^{-1}. The rhizobial isolates were also found to be effective in nodulating 21-day old seedlings grown in potting soil and irrigated with saline water of up to 12 dS m^{-1} after inoculation. The tolerance to high levels of salinity and the survival and persistence in severe and harsh desert conditions make these rhizobia highly valuable inoculums to improve productivity of the leguminous plants cultivated under extreme environments.

Key words: Desert soils, Lablab, Pigeonpea, Rhizobia, Salinity tolerance, Sesbania, United Arab Emirates

Introduction

Biological nitrogen fixation is an efficient source of nitrogen in the biosphere. Leguminous plants through their symbiotic relationship with certain gram-negative soil bacteria, collectively known as rhizobia, help to fix atmospheric nitrogen. Herridge et al. (2008) estimated that about 21 Tg of nitrogen is fixed annually through the crop legume-rhizobia symbiosis. The bacteria form nodules on the roots or rarely on the stem of legume hosts and by fixing atmospheric nitrogen into ammonia, they provide an easy and inexpensive way to enhance soil fertility and agricultural productivity. However, a number of factors affect the rhizobium-legume symbiotic relationship. These include the host symbiont compatibility and the physicochemical conditions of the soil, especially salinity and soil pH, nutrient deficiency, mineral and heavy metal toxicity, temperature extremes, insufficient or excessive soil moisture, etc. (Brockwell et al., 1995; Thies et al., 1995). Salinity in particular adversely affects the survival and proliferation of *Rhizobium* spp. in soil and rhizosphere, in addition to reducing plant growth, photosynthesis and demand for nitrogen from host plant. However, rhizobial populations are known to vary in their tolerance to major environmental factors (Wei et al., 2008). Singleton et al. (1982) showed that rhizobium strains can grow and survive at salt concentrations which are inhibitory to most agricultural legumes. Inoculation of such strains would enhance the nodulation and nitrogen fixing ability of the leguminous plants growing under saline conditions (Zahran, 1999; Ali et al., 2009). Furthermore, the ability of legume hosts to grow and survive in saline soils was also shown to improve when they were inoculated with salt-tolerant strains of rhizobia (Zou et al., 1995; Shamseldin and Werner, 2005).

The Arabian Peninsula is one of the driest and hottest regions in the world. The soils reflect the aridity of the climate - most being poorly developed, shallow and rich in lime, gypsum or salts. The region also lacks major river systems and many countries within it depend almost entirely on groundwater to irrigate farms. In several countries, an increase in farming area and large-scale extraction has depleted the groundwater reserves faster than the aquifer recharge from scanty rainfall.

Received 09 June 2011; Revised 29 May 2012; Accepted 01 July 2012; Published Online 28 November 2012

*Corresponding Author

N. Kameswara Rao
International Center for Biosaline Agriculture (ICBA), P.O. Box 14660, Dubai, United Arab Emirates

E-mail: n.rao@biosaline.org.ae

Making matters even more difficult, the growing urban areas are taking priority over the scarce freshwater, leaving agriculture to use low-value brackish or salty water with increased risk of soil salinization (Rao et al., 2009).

The naturally occurring soil rhizobia nodulating the leguminous plants in the desert regions are expected to have higher tolerance to prevailing adverse conditions such as salt stress, high temperatures and drought. For instance, Thrall et al. (2009) found that salt tolerance and growth were generally higher in rhizobial populations associated with *Acacia* spp., derived from saline soils than those from non-saline soils. Elanchezhian et al. (2009) reported tolerance to up to1000 mM of NaCl in *in v*itro studies of the rhizobium species associated with *Vigna marina*, a wild legume found growing in sandy seashores.

While nitrogen-fixing food and forage legumes tolerant of environmental stresses represents an important strategy to improve agricultural productivity, rhizobia with genetic potential for stress tolerance are equally vital for effective nodulation and enhanced productivity of the host-plants (Zahran, 1999). However, there are no previous reports on isolation and characterization of free living soils rhizobia from the desert soils of United Arab Emirates. In this paper, we present the results from a recent study to isolate and characterize the naturally occurring soil rhizobia nodulating different leguminous hosts.

Materials and Methods

Three leguminous species namely sesbania (*Sesbania sesban* (L.) Merrill), lablab (*Lablab purpureus* (L.) Sweet), and pigeonpea (*Cajanus cajan* (L.) Millsp.) showing root nodulation with free living soil rhizobia were used in this study. Nine-month-old plants of these species growing at ICBA research station (25°05'49"N and 55°23'25"E) were gently uprooted, the roots were harvested, washed and transported to the laboratory in plastic bags. The nodules were separated from the roots, followed by washing in sterile distilled water several times, surface sterilization with 70% ethanol for 10 seconds and then 0.1% mercuric chloride solution for 1-2 minutes. Four to five nodules were crushed on a glass slide in Phosphate Buffer, streaked onto yeast extract mannitol agar medium (YEMA) containing Congo-red dye in Petri plates and incubated at 28°C for 96 hours. The bacterial colonies were repeatedly sub-cultured by streaking on YEMA plates until pure cultures were obtained. The bacterial isolates were examined for Gram-staining reaction and other morphological characteristics as described by Somasegaran and Hoben (1994). The ability of eight isolates, namely PP1W1a, PP2WR1a and PP3PF1a from pigeonpea, LL2R2, LL4RW1 and LL6RP from lablab and SS2W1a and SS3RW2b from sesbania to tolerate salinity was studied under *in vitro* conditions. For this, the isolates were grown in Petri plates containing YEMA medium supplemented with different concentrations of sodium chloride (NaCl) to obtain saline culture media with electrical conductivities (EC) of 2, 4, 8, 10, 12, 14, 16, 20, 30 and 40 dS m^{-1}. The salinity tolerance of the rhizobial isolates was assessed by examining the plates after 24 hours of incubation for growth and morphological characteristics of the colonies. Gram stained slides of the rhizobial cultures were examined under compound microscope (magnification x 1000) for changes in cell morphology of rhizobia in response to salinity stress.

The effect of salinity on nodulation was studied by inoculating 21-day old pigeonpea, lablab and sesbania seedlings with yeast mannitol broth cultures, prepared according to Cappuccino and Sherman (2007). In additions to the three field legumes, seedlings of *Acacia ampliceps* Maslin, a leguminous tree species from Australia, were also inoculated to find the nodulating ability of the isolates. The seedlings were raised under greenhouse conditions (25-30°C) in 1 liter polyethylene pouches (pigeonpea and lablab) or small paper cups (sesbania and *A. ampliceps*), containing commercial potting soil (Van Egmond) and by irrigating with fresh water with a salinity of 2 dS m^{-1}. Prior to planting, sesbania seeds were scarified by soaking in concentrated H_2SO_4 for 1 hour, followed by repeated washing with distilled water. Five isolates – two from pigeonpea (PP1W1a and PP3PF1a), two from lablab (LL2R2 and LL4RW1) and one from Sesbania (SS3RW2b) were used for the inoculation study. The broth cultures, adjusted to an optical density of 0.5 (4.13 x 10^5 CFU ml^{-1}), were diluted by a factor of 2 with water before inoculation. Lablab seedlings were inoculated with 50 ml, sesbania and pigeonpea with 20 ml and *A. ampliceps* with 10 ml of diluted broth, proportional to the size of seedlings after 3-weeks of growth. The inoculation of seedlings was repeated the following day. For each rhizobial isolate, a total of 16 seedlings, divided into four sets each of four seedlings was inoculated. While one set of seedlings served as negative control (not inoculated), the other three inoculated sets were irrigated with saline water at electrical conductivities (EC) of 2, 6 and 12 dS m^{-1}, each

obtained from diluting saline ground water (~20 dS m^{-1}). The plants were harvested four weeks after inoculation and scored for the number of root nodules. In each treatment, the effectiveness of inoculation was determined from the mean number of nodules observed per plant and coded as: Effective (E) if the mean is higher than 5; Partially effective (P), if it is between 1 and 4; and Ineffective (I) if the mean number is <1. Since nodulation was ineffective in sesbania and absent in *A. ampliceps*, the nodulation data from only pigeonpea and lablab were analyzed statistically using GenStat (ver. 7.22 DE).

Results and Discussion

Nodule characteristics

The number of plants sampled per species, the location of nodules on the roots and the number of nodules per unit weight of the root systems together with the morphological data such as colour, shape, and size of the nodules are presented in Table 1. In both sesbania and pigeonpea the number of nodules was found to be proportional to the root mass. However, no such relationship was found in lablab. The nodules were light yellow to cream colored in all species. The shape was predominantly globose and semi-globose with smooth surface in sesbania, globose and semi-globose with striated surface in lablab, and semi-globose to irregular and coralloid in pigeonpea. While the nodules were found to be associated with primary and secondary roots in sesbania, they were found mostly on secondary roots in lablab and pigeonpea (Table 1). Mahmood and Iqbal (1994) observed that nodules in sesbania and lablab were globose with or without reticulate surface, elongated in pigeonpea and distributed both on primary and secondary roots in all. Nodules formed on primary roots are considered to be more efficient than those on the secondary roots and in cowpea, and a gradual shift was observed in the spatial distribution of nodules from primary roots to secondary roots under increased salt-levels (Predeepa and Ravindran, 2010).

Rhizobia isolation

The primary cultures isolated from sesbania and lablab showed a mixture of red, white and pink colored colonies. However, pigeonpea also showed slightly yellowish colonies in addition to the above colors. Pure cultures obtained from the primary culture showed varying characteristics in terms of color (white, pink and red), elevation (flat, raised), margins (entire, wavy, grainy and translucent) and colony size (1-10 mm) (Table 2). Twenty isolates were studied for colony morphology and Gram-staining reaction, among which eight isolates – three from pigeonpea (PP1W1a, PP2WR1a and PP3PF1a), three from lablab (LL2R2, LL4RW1 and LL6RP) and two from sesbania (SS2W1a and SS3RW2b) were identified as potential rhizobial strains.

In vitro test for salinity tolerance

Irrespective of the salt concentration of the YEMA medium, all the eight isolates grew within 24 hours. In lablab and sesbania, no significant differences were observed in colony size and morphology at different salt concentrations (Figure 1A-D). Microscopic observation of the Gram-stained slides of the cultures showed that some isolates underwent morphological changes in response to salt stress. Thus, the rhizobial cells in both the isolates of sesbania became shorter and more compact (Figure 2A, B). In the lablab isolate LL2R2, besides shortening of length, some cells became spherical at the high salinity (40 dS m^{-1}) (Figure 2C). In contrast to lablab and sesbania, the colonies from pigeonpea isolates were observed to be small with irregular margins but without any obvious changes to cell morphology at the higher salt concentration (40 dS m^{-1}). The rhizobial cells are known to respond to stress conditions by changing their morphology and size (Zahran, 1999). For instance, Kulkarni and Nautiyal (2000) found that in mesquite, the shape of temperature-stressed cells changed to spherical, compared with rod-shaped cells in non-stressed control.

Seedling inoculation test

In all the species, while the un-inoculated plants remained free of nodules, salinity had variable effect on the ability of the rhizobial isolates to infect and nodulate the host plants. For instance, pigeonpea showed consistent nodulation at all salinities (2, 6 and 12 dS m^{-1}), compared to the other legumes (Table 3). Lablab on the other hand, had a much higher level of nodulation than pigeonpea when irrigated with fresh water (2 dS m^{-1}), but showed a decrease with increase in salinity of the irrigation water. Sesbania showed slight nodulation up to 6 dS m^{-1} salinity but no nodules were observed at 12 dS m^{-1} salinity. Since the number of nodules formed is dependent on the compatibility of both the host plant and the infecting strains, it can be concluded that pigeonpea is a better host species than the others studied. The results also showed that the rhizobial isolates have considerable tolerance to salinity, confirming the observations from *in vitro* studies.

Table 1. Characteristics of nodules isolated from leguminous host-plants growing at a research farm in Dubai.

Host	Fresh weight of roots (g) Mean ± SD	No. of nodules Mean ± SD	Fresh weight of nodules (g) Mean ± SD	Weight of nodules/ weight of roots (g) Mean ± SD	Color	Shape and surface	Size (mm)	Location on roots
Sesbania	49.6±21.5	129.4±71.5	0.78±0.64	0.014±0.01	Cream	Globose, semi-globose, smooth surface	1-4	Primary and secondary roots
Lablab	34.1±24.3	21.0±10.7	0.50±0.40	0.037±0.06	Cream	Globose, semi-globose, striated	1-4	Secondary roots and tertiary roots
Pigeonpea	108. 8±26.8	64.7+74.5	2.01±2.57	0.016±0.02	Cream	Semi-globose, irregular and coralloid	1-2	Primary, secondary roots and tertiary roots

Table 2. Colony morphology of rhizobial isolates from three leguminous species.

Host	Isolate	Age of Culture (hr)	Color	Elevation	Size (mm)	Opacity	Margin
Sesbania	SS2W1a	120	White	Raised	2	Opaque	Wavy
	SS3RW2b	24	White	Raised	4	Translucent	Irregular
Lablab	LL2R2	120	Red	Raised	1	Opaque	Very smooth
	LL4RW1	24	White	Raised	5	Opaque	Very smooth, slight grainy appearance
	LL6RP	48	Pink	Raised	2.5	Opaque	Smooth wavy, grainy appearance
Pigeonpea	PP1W1a	72	White	Raised	10	Opaque	Grainy appearance
	PP2WR1a	72	White	Raised	9	Opaque	Wavy, grainy appearance, filamentous growth outside margin
	PP3PF1a	72	Pink	Raised	6	Opaque	Wavy

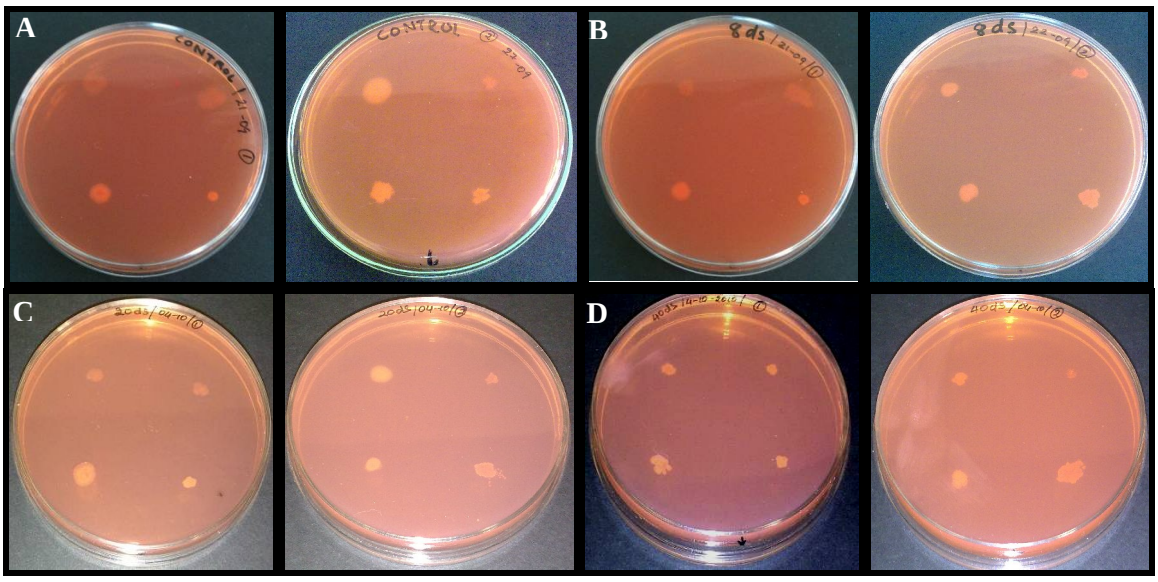


Figure 1. Rhizobial isolates growing on YEMA media supplemented with NaCl. Sets: A) Control, (B) 8 dS m^{-1}, (C) 20 dS m^{-1} and (D) 40 dS m^{-1}. Isolates: Sesbania (SS3RW2b, SS2W1a– upper two, left and right respectively in first plate of each set), lablab (LL2R2, LL4RW1 – lower two, left and right respectively in first plate and LL6RP – upper right in second plate of each set) and pigeon pea (PP1W1a, PP3PF1a, PP2WR1a – upper left, lower left and right respectively in second plate of each set).

In both pigeonpea and lablab, nodulation occurred with all the five isolates of rhizobia indicating their non-specific action. Also, the maximum number of nodules was observed when inoculated with rhizobia from other legumes than its own. In contrast to pigeonpea and lablab, sesbania showed ineffective nodulation, following inoculation. Interestingly, nodulation occurred with the two isolates from pigeonpea but not with its own, the reasons for which are not clear. *A. ampliceps* also did not show any nodulation, possibly because of cross-incompatibility of the rhizobia. In pigeonpea, the ability of different rhizobial isolates to nodulate the plants was found to be more or less similar. In lablab, although the differences in nodulating ability of the isolates were statistically insignificant, PP3PF1a and SS3RW2b exhibited more effectiveness than the others (P>0.05).

Legumes have immense value due to their capacity to enhance soil fertility by fixing atmospheric nitrogen through the symbiotic relationship with rhizobia. However, salinity, water deficit and temperatures stress are serious threats to rhizobium-legume symbiosis. Thus, while strategies to improve legume production in saline environments include selection of host genotypes that are tolerant to high salt conditions, inoculation with salt-tolerant strains of rhizobia could constitute another approach to improve legume productivity under symbiosis (Keneni et al., 2010). The rhizobia isolated in this study were able to grow at high salt concentration (40 dS m^{-1}) in *in vitro* cultures and formed nodules on seedlings irrigated with saline water at an EC of 12 dS m^{-1}.

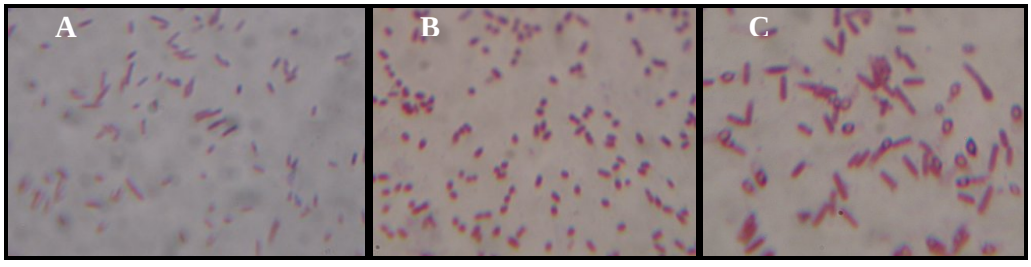


Figure 2. Changes in morphology of rhizobial cells in response to salinity. (A) Control (SS3RW2b), (B) 40 dS m^{-1} (SS3RW2b), (C) 40 dS m^{-1} (LL2R2) (x 1000).

Table 3. Effectiveness of rhizobial isolates in nodulating 21-day old seedlings of different leguminous species.

Plant	Salinity (dSm^{-1})	PP1W1a	PP3PF1a	LL2R2	LL4RW1	SS3RW2b
Sesbania	2	I	I	0	0	0
	6	I	0	I	0	I
	12	0	0	0	0	0
Lablab	2	E	P	E	E	E
	6	P	E	P	P	P
	12	I	P	I	P	P
Pigeonpea	2	P	P	P	P	P
	6	P	P	P	P	P
	12	P	P	P	P	P
A. ampliceps	2	0	0	0	0	0
	6	0	0	0	0	0
	12	0	0	0	0	0

E = effective, P = partially effective, I = ineffective and 0 = no nodulation.

Since the seed of the leguminous host-plants under study were not inoculated with rhizobia prior to sowing and there was no recent history of cultivation of other legumes in the same field, the rhizobia could be naturally occurring native strains probably associated with wild legumes such as *Indigofera colutea* and *Rhynchosia schimperi*, that grow as weeds in fallow fields. Although temperature tolerance of the rhizobial isolates was not investigated in this study, the fact that the rhizobia were isolated from the soils which experience high temperatures exceeding 50°C showed signs of their ability to survive and persist under extreme conditions. Another significant outcome of this study is that the isolated rhizobial strains did not seem to have any host specificity, which will be very beneficial when developing inoculants at a commercial level. Because of their natural adaptation to the harsh environmental conditions – especially salinity and heat stress, the rhizobial isolates will be highly beneficial as inoculums to improve productivity of compatible leguminous host plants grown in marginal environments.

Conclusions

Drought and salt-stress are the major constraints to plant productivity in desert environments and isolation of effective rhizobia to inoculate the leguminous crop plants could be an important strategy to improve the efficiency of rhizobium-legume symbiosis and thereby productivity. The results from this study showed that the rhizobia isolated from the desert soils are able to survive, grow and effectively nodulate their leguminous hosts even at high salt concentrations. Additional research to precisely identify the rhizobial strains through molecular characterization (16S-rDNA gene sequencing) and evaluate their growth performance, symbiotic efficiency and nodulating ability against other important environmental stresses such as temperature, pH and heavy metals is currently in progress.

Acknowledgements

We are grateful to Shawki Barghouti, Faisal Taha and Neeru Sood for their support. We would like to thank S. Rajeshwari and Salma Sheriff for helping in laboratory studies and M. Shahid for facilitating the field studies.

References

Ali, S. F., L. S. Rawat, M. K. Meghvansi and S. K. Mahna. 2009. Selection of stress-tolerant rhizobial isolates of wild legumes growing in dry regions of Rajasthan, India. ARPN J. Agric. Biol. Sci. 4:13-18.

Brockwell, J., P. J. Bottomly and J. E. Thies. 1995. Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. Plant Soil 174:143-150.

Cappuccino, J. G. and N. Sherman. 2007. Biochemical activities of microorganisms, In: Microbiology, A Laboratory Manual. pp. 143-203. The Benjamin-Cummings Publishing Co., California.

Elanchezhian, R., S. Rajalakshmi and V. Jayakumar. 2009. Salt tolerance of rhizobium species associated with *Vigna marina*. Indian J. Agr. Sci. 79:980-985.

Herridge, D. F., M. B. Peoples and R. M. Boddey. 2008. Global inputs of biological nitrogen fixation to agricultural systems. Plant Soil 311:1–18.

Keneni, A., F. Assefa and P. C. Prabu. 2010. Characterization of acid and salt-tolerant

rhizobial strains isolated from faba bean fields of Wollo, Northern Ethiopia. J. Agric. Sci. Technol. 12:365-376.

Kulkarni, S. and C. S. Nautiyal. 2000. Effects of salt and pH stress on temperature-tolerant Rhizobium sp. NBRI330 nodulating *Prosopis juliflora*. Curr. Microbiol. 40:221-226.

Mahmood, A. and P. Iqbal. 1994. Nodulation status of leguminous plants in Sindh. Pak. J. Bot. 26:7-20.

Predeepa, R. J. and D. A. Ravindran. 2010. Nodule formation, distribution and symbiotic efficacy of *Vigna ungiculata* L. under different soil salinity regimes. Emir. J. Food Agric. 22:275-284.

Rao, N. K., M. Shahid and A. S. Shabbir. 2009. Alternative crops for diversifying production systems in the Arabian Peninsula. Arab Gulf J. Sci. Res. 27:195-203.

Shamseldin, A. and D. Werner. 2005. High salt and high pH tolerance of new isolated *Rhizobium etli* strains from Egyptian soils. Curr. Microbiol. 53:1-7.

Singleton, P. W., S. A. El Swaift and B. B. Bohlool. 1982. Effect of salinity on *Rhizobium* growth and survival. Appl. Environ. Microb. 44:884-890.

Somasegaran, P. and H. J. Hoben.1994. The handbook of rhizobia, methods in legume-rhizobium technology. Springer Verlag, New York.

Thrall, P. H., L. M. Broadhurst, M. S. Hoque and D. J. Bagnall. 2009. Diversity and salt tolerance of native *Acacia* rhizobia isolated from saline and non-saline soils. Austr. Ecol. 34:950-963.

Thies, J. E., P. L. Woomer and P. W. Singleton. 1995. Enrichment of *Bradyrhizobium* spp. population in soil due to copping of homologous host legume. Soil Biol. Biochem. 27:633-636.

Wei, G. H., X. Y. Yang, Z. X. Zhang, Y. Z. Yang and K. Lindstrom. 2008. Strain *Mesorhizobium* sp. CCNWGX035; A stress tolerant isolate from *Glycyrriza glabra* displaying a wide host range of nodulation. Pedosphere 18:102-112.

Zou, N., P. J. Dart and N. E. Marcar. 1995. interaction of salinity and rhizobial strain on growth and N2-fixation by *Acacia ampliceps.* Soil Biol. Biochem. 27:409-413.

Zahran, H. H. 1999. *Rhizobium*-legume sysmbiosis and nitrogen fixation under severe conditions and in arid climates. Microbiol. Mol. Biol. R. 63:968-989.

Emir. J. Food Agric. 2013. 25 (2): 109-116
doi: 10.9755/ejfa.v25i2.7151
http://www.ejfa.info/

PLANT SCIENCE

Agronomic and physiological responses of pearl millet ecotype (*Pennisetum glaucum* (L.) R. Br.) to saline irrigation

Leila Radhouane*

National Research Institute for Agricultural of Tunisia, Avenue Hédi Karray, 2049 Ariana, Tunisie

Abstract

The objective of this study was to identify morphological and physiological traits for salinity tolerance in Tunisian autochthonous ZZ pearl millet ecotype (*Pennisetum glaucum* (L.) R. Br) under local conditions. ZZ ecotype was cultivated under different levels of salinity and growth parameters, water relations and mineral content were measured. Results showed that ZZ pearl millet ecotype was unable to store the large amounts of salt in the leaves, while maintaining high leaf water content and without a grave consequent on panicle yield. This ecotype is fitted for a selective sodium sequestration in the vacuole.

Key words: pearl millet, Saline water, Morphological trait, Physiological trait, Leaf water content, Ion content

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br] is one of the major cereal crops of the semi-arid regions of Africa and Asia and it is certainly the mainstay for millions of people in the Sahel. It's grown as grain and fodder crop (Blummel et al., 2003). In Tunisia, pear millet is not the staple food of rural populations as in the other countries of Africa. Nevertheless, it occupies a very important part of surfaces every year in the centre and in the South of the country. In 2003, local production was about 19150 metric tonnes of pearl millet grains principally produced in Kairouan (50%), Medenine (26%), Nabeul (15.6%) and Mahdia (3.4%) regions (FAO, 2003). All pearl millet production is used for a variety of food products.

Pearl millet is a summer irrigated crop. However, Tunisia, as in the majority of the arid areas, is classified among the countries threatened by dryness and salinity (Qadir et al., 2006). In fact, water availability is below the threshold of 1000 m^3/person/year (Paranychianakis and Chartzoulakis, 2005). In order to overcome water scarcity, many countries have adopted the use of marginal water for irrigation (Oron et al., 2002). However, the salinity of those water sources typically exceeds the limit tolerated by conventional crop plants which are for the majority sensitive glycophytes (Hu et al., 2005).

Plants, whether glycophyte or halophyte, cannot tolerate large amounts of salt in the cytoplasm, so, they develop a plethora of mechanisms to cope with salt stress and to facilitate their metabolic functions (Zhu, 2003). In fact, salt stress affects all the major processes such as growth, photosynthesis, protein synthesis, and energy and lipid metabolism (Parida and Das, 2005). However, some moderately or highly salt tolerant plants can survive in salty environments. These species are able to avoid ion toxicity and maintain water uptake in the presence of high salt concentrations (Munns, 2002).

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is rated to be fairly tolerant to salinity (Krishnamurthy et al., 2007). Moreover, availability of high levels of tolerance in other species of *Pennisetum* (Muscolo et al. 2003) and within the *P. glaucum* (Krishnamurthy et al., 2007) offers a scope for understanding the traits related to tolerance and to integrate these tolerant crop species/genotypes into appropriate management programs to improve the productivity of the saline soils (Baisakh et al., 2008).

Identifying autochthonous ecotypes growing under local agricultural conditions with significant levels of beneficial factors may promote the value-added cultivation and enhancing the agricultural economy. The effects of salt stress on plant growth and physiology have been well documented in other

Received 23 January 2012; Revised 01 April 2012; Accepted 06 May 2012; Published Online 28 November 2012

*Corresponding Author

Leila Radhouane
National Research Institute for Agricultural of Tunisia, Avenue Hédi Karray, 2049 Ariana, Tunisie

Email: radhouane.leila@iresa.agrinet.tn

cereals (Lopez et al., 2010; Mehta et al., 2010). However data on specific effects of salt stress in autochthonous pear millet are still fragmentary especially effects on pearl millet physiology. The research objective was to identify morphological and physiological traits for salinity tolerance in Tunisian autochthonous ZZ pearl millet ecotype under local conditions.

Materials and Methods

Plant material

Autochthonous ZZ pearl millet ecotype whose salt tolerance characteristics were determined in our previous study (Radhouane, 2008) was used. It is tall stature (> 2m) and has an intermediate duration of cycle (about 80 days). It was collected in Zarzis. The site is located at 33°30' latitude and 11°07' longitude. It has a Mediterranean climate.

Plant growth and treatments

The experiment was carried out at the farm of the Tunisian Agricultural Research Institute in Tunis during the cropping season of 2008. The site is located at 36°51' latitude and 10° 11' longitude. The soil of the experimental site was clay loam. It was sown at 22 May 2008 into randomized block design with four replications.

The sampling area was 5.5 m², having rows five meters long was used. Sowing was done in hills and row to row distance of 50 cm and hill to hill distance of 30 cm were used. A basal dose of 50 kg N in the form of ammonitrate 33% fertiliser was applied at sowing. Irrigation and all other agronomic practices were carried out uniformly for all the experimental units. Total irrigation volume of 420 mm was applied (one a week) and treatments were initiated at emergency of the fourth leaf.

Three salt levels were applied.

T_1: water containing 1 g/l NaCl (control no added NaCl)

T_2: T_o + 3 g NaCl = 4g/l

T_3: T_o + 6 g NaCl = 7g/l

Technical Analysis

Data were recorded on:

- plant height (PHT) in cm
- flag leaf surface (FLS) in cm²
- panicle grain yield (PGY) in g
- flag leaf water content (RWC) in %
  - leaf water potential (LWP) in (MPa)
  - some ions contents (Na^+, K^+, Ca^{2+}) in %

Plant height was determined using a graduated ruler (from the neck to the insertion of the panicle, while leaf area was measured with leaf area meter (MK 2) immediately after harvesting. At maturity, mature panicles were shelled and individually weighed.

Relative water content (RWC) was determined on flag leaf tissues excised in the morning (around 8:00 am). Excised leaves were measured for fresh weight (FW), and then rehydrated in a water-filled Petri dish at room temperature. Turgor weight (TW) was measured by allowing full rehydration (16 h), removing all water on the leaf surface, weighing, and then leaves were dried at 70°C for 48h to determine DW (Hensen, 1982). The relative water content was calculated from the following equation

[RWC = 100[(FW - DW)/(TW - DW)].

Leaf water potential was measured at the abaxial surface of intact plants with pressure chamber (Scholander et al., 1965).

For determining ion content**,** mature flag leaves were taken and oven dried for 72h at 70°C. After desiccation, samples were minced and incubated overnight in a 0.1N HNO3. After filtering, 0.5ml of the solution was used for determination ion contents (Na^+, K^+, Ca^{2+}) by flame photometry (Model 410, Corning, England) (Gulati and Jaiwal, 1992).

Statistical analysis

Data regarding plant height, flag surface leaf, panicle grain yield, relative water content and ions content were recorded on 50 plants at time of maturity.

Data were statistically analyzed using analysis of variance techniques appropriate for randomized complete block design. Main and interaction effects were separated by LSD test at 0.05 level of probability, if the F-values were significant.

Results and Discussion

The detrimental effects of high salinity on plants can be observed at the whole-plant level as the death of plants and/or decreases in productivity (Parida and Das, 2005).

Salinity is known to affect also various facets of plant metabolism. In fact, the various concentrations of NaCl had a significant effect on ZZ pearl millet ecotype behaviour.

Plant height

The statistical analysis of the data indicated that salinity had significant effect on plant height of autochthonous ZZ pearl millet ecotype (Fig.1). PHT value decreased significantly by salt treatments. Maximum plant height of 207cm was attained by control (T_1) and plants treated with T_3 had the lowest mean PHT of about 169 cm. Plant

height for T_3 was 18% significantly lower ($P \leq 0.01$) than T_1 and 13% than T_2.

Generally, salinity stress results in a clear stunting of plants (Takemura et al., 2000). Slower growth is a general adaptive feature for plant survival under stress, allowing re-directing cell resources (e.g., energy and metabolic precursors) towards the defence reactions against stress (Zhu, 2001).

In fact, salt in soil water inhibits plants ability to take up water, and this leads to slower growth (Manchanda and Neera, 2008). Suppression of growth occurs in all plants, but their tolerance levels and rates of growth reduction at lethal concentrations of salt vary widely among different plant species. Processes that regulate growth reduction have not been well documented (Hasegawa et al., 2000).

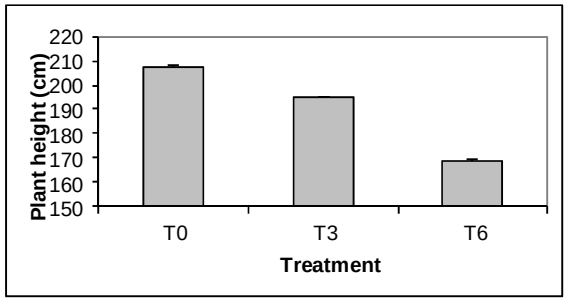


Figure 1. Plant height of ZZ pearl millet ecotype dependent on salinity treatment.

Flag leaf surface

Increasing NaCl concentration resulted in reduced leaf size for ZZ ecotype (Figure 2). ZZ pearl millet ecotype had maximum flag leaf area at T_0 treatment. Leaf area for T_3 was 6.5 % significantly lower ($P \leq 0.01$) than T_1 and 2.5% than T_2.

Muscolo et al. (2003) reported that *Panicum clandestinum* growth and leaf length decreased with increase in salinity.

The decreased rate of leaf growth after an increase in soil salinity is primarily due to the osmotic effect of the salt around the roots (Passioura and Munns, 2000). Salt stress initially inhibits leaf expansion through reduced turgor and may in fact eventually result in increased cell wall extensibility, which counteracts the negative effects of low turgor. In the presence of salt, cell wall extensibility of the growing region may decrease (Nonami et al., 1995).

The reduction in leaf growth must be regulated by long distance signals in the form of hormones or their precursors. It's independent of carbohydrate supply (Munns et al., 2000) and water status (Frickle and Peters, 2002).

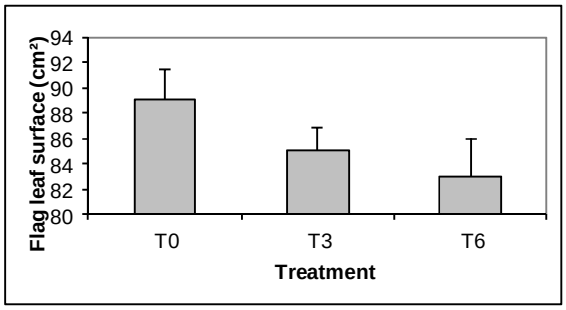


Figure 2. Flag leaf surface of ZZ pearl millet ecotype dependent on salinity treatment.

Panicle grain yield

Grain head yield of ZZ ecotype continuously decreased with increasing salinity (Figure 3). The lowest head yield for ZZ ecotype occurred with T_3 treatment. Panicle grain yield declined by 8.7 % as planting was effectuated with high salinity. Nerveless, RGC reduction was about 1.8% when T_2 treatment was applied.

Salinity is the major environmental factor limiting plant growth and productivity (Allakhverdiev et al., 2000). The altered water status leads to initial growth reduction and limitation of plant productivity (Parida and Das, 2005).

Salt stress affects uptake, transport and utilization of different nutrients (Grattan and Grieve, 1999), which may results in excessive accumulation of Na^+ and Cl in tissue (Saqib et al., 2005) and ultimately reduction in crop yield.

Pearl millet grain yields were slightly affected by moderate saline irrigation. This result is corroborated by Hussain et al. (2008). Depressed photosynthesis has been suggested to be responsible for at least part of the growth and yield reduction (Munns, 2002).

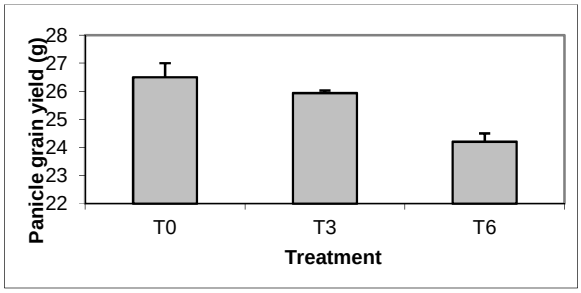


Figure 3. Panicle grain yield of ZZ pearl millet ecotype dependent on salinity treatment.

Leaf water content

Within a salinity level, differences in RWC were not significant (P≤ 0.01) with a range of 84-87% of saturated water content (Figure 4). Relative water content was statistically similar to that of the control. Similar result was found in RWC of many plants (Rivelli, 2002).

Lu et al. (2002) showed that RWC remained relatively unchanged under salinity for *Sueda salsa*. Maintenance of favourable plant water status contributes to salinity tolerance of the salt tolerant ecotypes (Oweis, 2009).

Maintaining a high water content in the growing leaves and in leaves expansion in the presence of stress, indicates osmotic adjustment effectiveness (Meloni et al., 2004). The osmotic adjustment (if any) results in a slower decrease of RWC when the leaf water potential continues to decline as observed by some authors on *T. durum* and *T. polonicum* (Al Hakimi et al., 1995).

ZZ pearl millet ecotype was able to balance the low external water potential and may potentially generated turgor and growth by many mechanisms to protect sensitive cellular sites of the salt adverse effects. This ZZ ecotype performance suggests a tolerance to salinity.

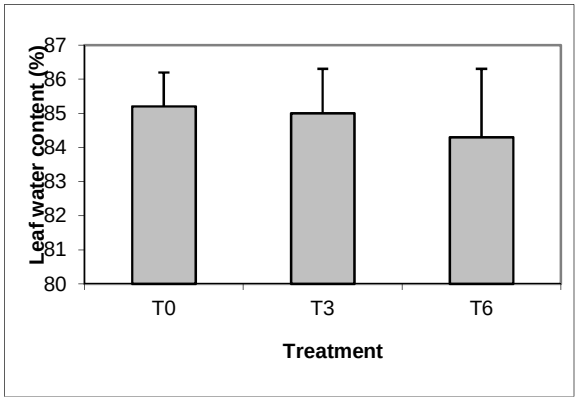


Figure 4. Leaf water content of ZZ pearl millet ecotype dependent on salinity treatment.

Leaf water potential

The leaf water potential (ψ flag leaf) of ZZ pearl millet ecotype was higher in control plants as compared to the two different treatments (Figure 5). Brackish water irrigation has reduced water potential of 28% and 37% respectively for moderate treatment (T_2) and severe stress (T_3). Water potential becomes more negative with an increase in salinity (Gulzar et al., 2003). Decrease of the leaf water potential under salt stress has been reported by many authors, especially in C_4 (Poaceae) (Maricle et al., 2006).

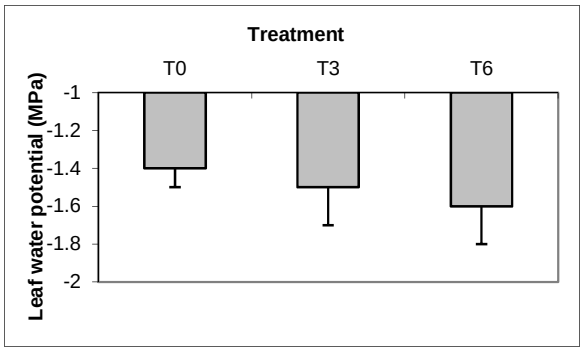


Figure 5. Leaf water potential of ZZ pearl millet ecotype dependent on salinity treatment.

Water potential reduction is the result of a rapid osmotic adjustment and an increase of the concentrations of osmotically (Koyro, 2006).

Antagonic interactions

Saline environment, most commonly mediated by high NaCl, results in perturbation of ionic steady state not only for Na^+ and Cl^- but also for K^+ and Ca^{2+} (Niu et al., 1995). Plants showed a change of the mineral composition towards Na^+ and Cl^- uptake especially in the leaf (Koyro, 2006).

Accumulation of Na^+, K^+, Ca^{2+} ions in flag leaf of ZZ pearl millet ecotype under three NaCl concentrations were presented in Figures 6, 7 and 8.

Na^+ is the predominate soluble cation in many of the soils of arid and semi-arid areas (Zhu et al., al., 2004). When saline applications were made, there has been a Na^+ ion increasing in the leaves of ZZ ecotype resulting in positive correlations between leaf Na content and NaCl (Figure 6).

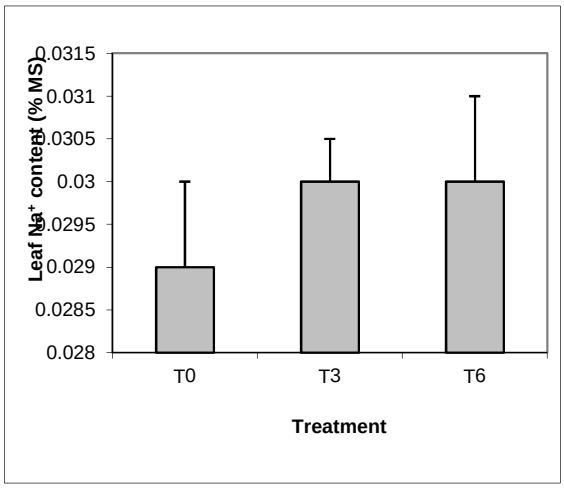


Figure 6. Foliar Na+ content of ZZ pearl millet ecotype dependent on salinity treatment.

As in some other plants (Kusvuran et al., 2007), tolerance to salinity is has been related to Na^+ ion accumulation in plant green matter.

ZZ pear millet ecotype which grows and survives in saline media is fitted for a selective sodium sequestration in the vacuole (Cuin et al., 2003). This system therefore functions as a metabolic regulatory cycle to avoid critical concentrations in the cell. This adaptive mechanism thus has a homeostatic function in supplying metabolism with essential elements as well as detoxifying function (Smekens and Tienderen, 2001).

Sodium sequestration into vacuole appears to constitute the most effective mechanism of plant cells to handle efficiently high concentrations of salts and to prevent their toxic effects on cytoplasm. The compartmentalization of Na^+ into vacuoles allows plants to use Na^+ as an osmoticum, maintaining the osmotic potential that increases the water content within the cells (Blumwald et al., 2000).

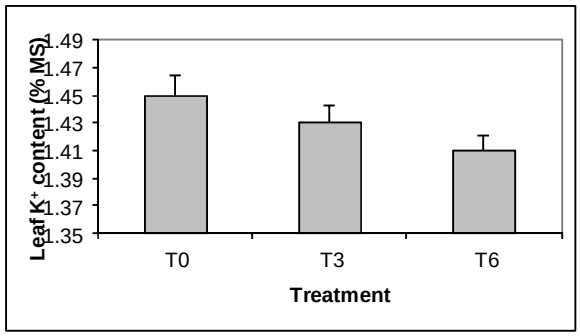


Figure 7. Foliar K+ content of ZZ pearl millet ecotype dependent on salinity treatment.

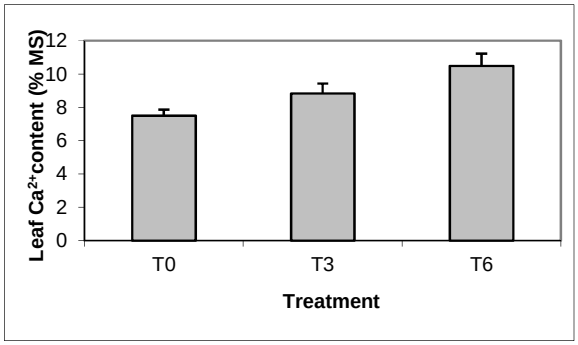


Figure 8. Foliar Ca2+ content of ZZ pearl millet ecotype dependent on salinity treatment.

Na^+ compartmentation is regulated by Na^+/H^+ antiporters (Hasegawa et al., 2000). The overexpression of genes encoding Na^+/H^+ antiporters in different plant species induced the tolerance of plants to salinity (Zhang and Blumwald, 2001).

Potassium (K^+) concentration in mature leaves was significantly ($p<0.05$) lower in plants grown with salinity (Figure 7). Although it has been found that there were increases in Na^+ ion intake, there has been a decrease in K^+ ion intake, External Na^+ negatively impacts intracellular K^+ influx, attenuating acquisition of this essential nutrient by cells (Niu and al., 1995). It has been reported that leaf potassium concentration is lowered by increasing NaCl concentration (Ozalp et al., 2000). Under saline soils, higher levels of external Na^+ interfere with K^+ acquisition limiting plant K uptake **(**Hussain et al., 2008).

Liu et al. (2000) reported that high affinity K^+ transporters may act as low affinity Na^+ transporters under salt stress which may reduce K^+ uptake. In the cytosol, the presence of K^+ is essential for the activation of many enzymes, for example, those involved in pyruvate synthesis and protein translation. Due to physicochemical similarities between Na^+ and K^+, excess Na^+ tends to substitute K^+, for Na^+ at these binding sites and hence impair cellular biochemistry (Manchanda and Neera, 2008).

Saline water irrigation has increased Calcium content in the leaf of ZZ ecotype (Fig.8) of 17% and 40% respectively for moderate treatment (T_2) and severe stress (T_3). Similar result was found by Munns and Tester (2008).

Calcium has been shown to ameliorate the adverse effects of salinity on plants and is well known to have regulatory roles in metabolism (Ehret et al., 1990)

Bush (1995) hypothesized that sodium ions may compete with calcium ions for membrane-binding sites. Therefore, high calcium levels can protect the cell membrane from the adverse effects of salinity .Increase of Ca^{2+} uptake is associated with the rise of ABA under salt stress and thus contributes to membrane integrity maintenance, which enables plants to regulate uptake and transport under high levels of external salinity in the longer term (Chen et al., 2001).

Conclusion

Autochthonous ZZ pearl millet is an ecotype known to be salt tolerant in the south of Tunisia. The ability of this ecotype to cope with severe salt stress is the combined characteristic of many plant features, both morphological and physiological. These mechanisms enable ZZ pearl millet ecotype to store the large amounts of salt in the leaves, while maintaining high leaf water content and

without a grave consequent on panicle yield. ZZ pearl millet ecotype is enabling to be a widely distributed species on all continents especially near the sea and to be utilized for different applied purposes: production of biomass for energy, enzymes or antioxidants and phytorremediation programs of saline soils.

References

Allakhverdiev, S. I., A. Sakamoto, Y. Nishiyama, M. Inaba and N. Murata. 2000. Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in Synechococcus sp. Plant Physiol. 123:1047–1056.

Baisakh, N., K. S. Prasanta and P. Varadwaj, 2008. Primary responses to salt stress in a halophyte, smooth cordgrass (*Spartina alterniflora Loisel*.). Funct. Integr. Genom. 8:287–300.

Blummel, M., E. Zerbini, B. V. S. Reddy, C. T. Hash, F. Bidinger and A. A. Khan. 2003. Improving the production and utilization of sorghum and pearl millet as livestock feed: progress towards dual-purpose genotypes. Field Crops Res. 84:143-158.

Blumwald, E. 2000. Sodium transport and salt tolerance in plants. Curr. Opin. Cell Biol. 12(4):431-434.

Chen, S., J. Li, S. Wang, A. Huttermann and A. Altman. 2001. Salt, nutrient uptake and transport, and ABA of *Populus euphratica*; a hybrid in response to increasing soil NaCl. Trees-Struct. Funct. 15:186–194.

Cuin, T. A., A. J. Miller, S. A. Laurie and R. A. Leigh. 2003. Potassium activities in cell compartments of salt-grown barley leaves. J. Exp. Bot. 54:657-661.

Ehret, D. L., B. L. RemannHarvey and A. Cipywnyk. 1990. Salinity-induced calcium deficiencies in wheat and barley. Plant Soil 128:143–151.

FAO 2003. La production et la surface du mil dans certains pays et dans le monde. Statistics Series 57(177):1-10.

Fricke, W. and W. S. Peters. 2002. The biophysics of leaf growth in salt-stressed barley. A study at the cell level. Plant Physiol. 129:374-88.

Gratten, S. R. and C. M. Grieve. 1999. Salinity mineral nutrient relation in horticultural crops. Sci. Hort. 78:127-157.

Gulati, A. and P. K. Jaiwal. 1992. Comparative salt responses of callus cultures of Vigna radiata (L.) wilczek to various osmotic and ionic stresses. J. Plant Physiol. 141:120-124.

Gulzar, S., M. A. Khan and I. A. Ungar. 2003. Salt tolerance of a coastal salt marsh grass. Commun. Soil Sci. Plant Anal. 34:2595–2605.

Hakimi, A., P. Monneveux and G. Galiba. 1995. Soluble sugars, proline and relative water content (RWC) as traits for improving drought tolerance and divergent selection for RWC from *Triticum polonicum* into *Triticum durum*. J. Genet. Breed. 49:237-244.

Hasegawa, P. M., R. A. Bressan, J. K. Zhu and H. J. Bohnert. 2000. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51:463-499.

Hensen, I. E. 1982. Osmotic adjustment to water stress in pear millet (*Pennisetum americanum* (L.) Leake) in a controlled environment. J. Exp. Bot. 33(132):78-87.

Hu, Y., W. Fricke and U. Schmidhalter. 2005. Salinity and the growth of non-halophytic grass leaves: the role of mineral nutrient distribution. Funct. Plant Biol. 32:973-985.

Hussain, K., M. Ashraf and M. Y Ashraf. 2008. Relationship between growth and ion relation in pearl millet (*Pennisetum glaucum* (L.) R. Br.) at different growth stages under salt stress. Afr. J. Plant Sci. 2(3):23-27.

Koyro, H. W. 2006. Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L.). Environ. Exp. Bot. 56(2):136-146.

Krishnamurthy, L, R. Serraj, K.N. Rai, C.T. Hash and A.J. Dakheel, 2007. Identification of pearl millet [*Pennisetum glaucum* (L.) R. Br.] lines tolerant to soil salinity. *Euphytica,* 158: 179-188.

Kusvuran, S., F. Yasar, S. Ellialtioglu and K. Abak. 2007. Utilizing Some of Screening methots in order to determine of tolerance of salt stress in the melon (*Cucumis melo* L.). Res. J. Agric. Biol. Sci. 3(1):40-45.

Liu, W., D. P. Schachtman and W. Zhang. 2000. Partial deletionof a loop region in the high affinity K+ transporter HKT1 changes ionic permeability leading to increased salt tolerance. J. Biol. Chem. 275:27924-27932.

López, P. U., A. Robredo, M. Lacuesta, A. Muñoz-Rueda and A. Mena-Petite. 2010. Atmospheric CO_2 concentration influences the contribution of osmolyte accumulation and cell wall elasticity to salt tolerance in barley cultivars. J. Plant Physiol. 167(1):15-22.

Lu, C. M., N. W. Qiu, Q. T. Lu, B. S. Wangand and T. Y. Kuang. 2002. Does salt stress lead to increased susceptibility of Photosystem II to photoinhibition and changes in photosynthetic pigment composition in halophyte suaeda salsa grown outdoors? Plant Sci. 163:1063-1068.

Manchanda, G. and G. Neera. 2008. Salinity and its effects on the functional biology of legumes. Acta Physiol. Plant. 30:595–618.

Maricle, B. R., R. C. Douglas, C. S. Campbell 2007. Biophysical and morphological leaf adaptations to drought and salinity in salt marsh grasses. Env. Exp. Bot. 60(3):458-467.

Mehta, P., A. Jajoo, S. Mathur and S. Bharti. 2010. Chlorophyll a fluorescence study revealing effects of high salt stress on Photosystem II in wheat leaves. Plant Physiol. Biochem. 48(1):16-20.

Meloni, D. A., M. R. Gulotta, C. A. Martinez and M. A. Oliva. 2004. The effects of salt stress on growth, nitrate reduction and proline and glycinebetaine accumulation in *Prosopis alba*. Braz. J. Plant Physiol. 16(1):39- 46.

Munns, R. 2002. Comparative physiology of salt and water stress. Plant Cell Environ. 25:239-250.

Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59:651–81.

Munns, R., J. Guo, J. B. Passioura and G. R. Cramer. 2000. Leaf water status controls day-time but not daily rates of leaf expansion in salt-treated barley. Aust. J. Plant Physiol. 27:949-5790.

Muscolo, A., M. R. Panuccio and M. Sidari. 2003. Effects of salinity on growth, carbohydrate metabolism and nutritive properties of kikuyu grass (*Pennisetum clandestinum* Hochst). Plant Sci. 104:1103-1110.

Niu, X., R. A. Bressan, P. M. Hasegawaand and J. M. Pardo. 1995. Ion homeostasis in NaCl stress environments. Plant Physiol. 109:735-742.

Nonami, H., K. Tanimoto, A. Tabuchi, T. Fukwjama and Y. Hashimoto. 1995. Salt stress under hydroponic conditions causes changes in cell wall extension during growth. Acta Hort. 396:91–98.

Oron, G., L. DeMalachGillerman, I. David and S. Lurie. 2002. Effect of water salinity and irrigation technology on yield and quality of pears. Biosyst. Eng. 81:237-247.

Oweis, M. 2009. Durum wheat and barley productivity in saline–drought environments. Europ. J. Agr. 31(1):1-9.

Ozalp, V. C., A. Oktem, S. M. Saqlan Naqvi and M. Yu¨ cel. 2002. Photosystem II and cellular membrane stability evaluation in hexaploid wheat seedlings under salt stress conditions. J. Plant Nutr. 23(2):275–283.

Paranychianakis, N. V. and K. S. Chartzoulakis, 2005. Irrigation of Mediterranean crops with saline water: from physiology to management practices. Agric. Ecosys. Environ. 106:171-187.

Parida, A. K. and A. D. Das. 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicol. Environ. Safety 60:324-349.

Passioura, J. B. and R. Munns. 2000. Rapid environmental changes that affect leaf water status induce transient surges or pauses in leaf expansion rate. Aust. J. Plant Physiol. 27:941-48.

Qadir, M., S. Schubert, A. D. Noble, M. Saqib and M. Saifullah. 2006. Amelioration strategies for salinity-induced land degradation. CAB Reviews: Perspec. Agric. Veterin. Sci. Nutr. Natur. Resour.1(69):1-12.

Radhouane, L. 2008. Effet du stress salin sur la germination, la croissance et la production en grains chez quelques écotypes de mil (*Pennisetum glaucum* L. R. Br.) autochtones de Tunisie. Comptes Rendus Biol. 331(4):278-286.

Rivelli, P. 2002. Water potential in crop. I. The soil water balance. Aust. J. Agric. Res. 54:329-336.

Saqib, M., J. Akhtar and R. H. Qureshi. 2005. Na exclusion and salt resistance of wheat (*Tricticum aestivum*) in saline-waterlogged

conditions are improved by the development of adventitious nodal roots and cortical root aerenchyma. Plant Sci. 169:125-130.

Scholander, P. F., H. T. Humme, E. D. Bradstreet and A. Hennigsen. 1965. Sap pressure in vascular plants. Science 148:339-346.

Smekens, M. J. and P. H. Tienderen. 2001. Genetic variation and plasticity of *Plantago coronopus* under saline conditions. Acta Oecol. 22:187–200.

Takemura, T., N. Hanagata, K. Sugihara, S. Baba, I. Karube and Z. Dubinsky. 2000. Physiological and biochemical responses to salt stress in the mangrove, *Bruguiera gymnorrhiza*. Aquat. Bot. 68:15-28.

Zhang, H. X. and E. Blumward. 2001. Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. Nat. Biotechl. 9:765-768.

Zhu, J. K. 2001. Plant salt tolerance. Trends Plant Sci. 6:66-72.

Zhu, J. K. 2003. Regulation of ion homeostasis under salt stress. Curr. Opin. Plant Biol. 6:441-445.

Zhu, R. A. Bressan, P. M. Hasegawa, Y. Zhao and H. Zhang. 2004. Expressed sequence tags from Thellungiella halophila, a new model to study plant salt-tolerance. Plant Sci. 166: 609-614.

Emir. J. Food Agric. 2013. 25 (2): 117-123
doi: 10.9755/ejfa.v25i2.10655
http://www.ejfa.info/

PLANT SCIENCE

Yield performance and responses studies of chickpea (*Cicer arietinum* L.) genotypes under drought stress

Muhammad Yaqoob[1*], Phill A. Hollington[2], Ahmad B. Mahar[3] and Zulfiqar A. Gurmani[4]

[1]*Arid Zone Research Institute, Ratta Kulachi, D. I. Khan, Pakistan*
[2]*Centre of Arid Zone Research University of Wales, Bangor, United Kingdom*
[3]*National Agricultural Research Centre, P.O. N.I.H., Park Road, Islamabad, Pakistan*
[4]*Fodder Program, NARC, Islamabad, Pakistan*

Abstract

Drought is a major abiotic constraint limiting the chickpea yield up to a greater level in Pakistan. The yield level remain very low under prolong moisture deficit conditions. A set of 40 chickpea lines including some approved varieties were assessed for drought tolerance under glasshouse conditions. The crop was given an artificial drought stress at pre-flowering stage for a period of 30 days and then re-irrigated regularly till harvesting. The observations were recorded on plant wilting-I, plant wilting-II, crop recovery percentage and grain yield. The results revealed a relatively divergent response of various chickpea lines/varieties for all the studied parameters. Genotypes SL-05-30 NCS950219 and F-97-112 C remained superior while observing plant wilting-I by scoring 1, 1.67 and 2.00 at 1-9 rating scale and produced higher yield of 18.43, 18.33 and 18.23 grams per plant respectively. The plant recovery response revealed that more than 50% of studied lines could not properly recover after termination of moisture stress suggesting that moisture stress at pre-flowering has usually lethal effect on chickpea plants. Kabuli type chickpea lines were more sensitive to moisture stress and high temperature and produced lower yields as compared to desi type chickpea.

Key words: Chickpea germ plasm screening, Drought stress, Plant wilting, Plant recovery and grain yield

Introduction

Chickpea is the major pulse crop of Pakistan, which is grown on about one million hectare in the country each year (MINFAL, 2009). More than 95% production of chickpea comes from rainfed areas where the crop is sown on residual moisture after monsoon. Among various factors minimizing the crop yield, the terminal drought stress is most often, which reduces the crop yield up to greater extent. Field screening of chickpea germplasm against drought stress is usually not reliable because of uncertain rainfall at critical stage of artificial moisture stress imposed to the crop.

Chickpea with indeterminate habit grows well under favourable conditions. The crop needs variable temperature during its various growth phases. If grown under favourable moisture, it produces higher pods and grain yield under heat and drought in the later part of it reproductive stage. A significant pod abortion has been observed under sever moisture stress especially during commencement of pod set (Leport et al., 1999). The drought tolerance in their case was found to be directly proportional to deep root system and high leaf water potential (LWP). High temperature stress also causes yield losses because of damage to reproductive organs (Anyia and Herzy, 2000) and had reduced total dry matter and grain yield during drought (Leport et al., 2006). Singh et al. (1997), Kashiwagi et al. (2005) and Lopez et al. (2006) have screened out some pulses germ plasm accessions with greater genetic variability in various traits. They also identified some drought tolerant related characteristics in the chickpea plant. A multipinnate chickpea lines reduce amount of energy stored in leaf due to high level of reflection Moisture stress to chickpea is more important at pre-flowering stag, because it is the most damaging stage to yield and yield parameters and plant needs abundant availability of moisture in the root zone. Therefore, artificial stress at this stage will lead to screening of drought resistant genotypes in chickpea (Leport, 2006). Aslam et al. (2008) evaluated the performance of 23 chickpea

Received 23 January 2012; Revised 01 April 2012; Accepted 06 May 2012; Published Online 28 November 2012

*Corresponding Author

Muhammad Yaqoob
Arid Zone Research Institute, Ratta Kulachi, D. I. Khan, Pakistan

Email: yaqoobawan313@gmail.com

genotypes and reported significant variation in morphological, physiological, phonological characters and yield and yield components. In case of breeding drought and heat-resistant chickpea days to the first flowering and maturity should be evaluated ahead of many other phenological traits, harvest index, biological yield and pods per plant to escape terminal drought and heat stresses for increased yield (Canci and Tokar, 2009) Karadavut et al. (2010) studied the some fababean genotypes across the environments and reported significant variation in yield among genotypes and also over environments. Khan et al. (2010) studies genetic variability and correlation in wheat under water stress conditions. They reported significant genetic variability in various morphological and physiological traits. Shamsi et al. (2010) observed significant difference in most of the yield components when irrigated at flowering and pod filling stage in chickpea. They also suggested that pod-filling is the most sensitive stage to drought stress, and under water limitation conditions, that can considerably increase grain yield at this stage by one time irrigation

The present studies are actual screening of chickpea germ plasm based on tangible selection criterion being output of two different experiments under drought stress environment. The initial studies on development of field screening technique was carried out by Yaqoob et al. (2011) suggesting that moisture stress at pre-flowering stage being harmful and detrimental is the, most critical for screening chickpea germ plasm under drought prone conditions. These studies were further endorsed by Yaqoob et al. (2012) through their studies on root and shoot behaviors of chickpea genotypes under different moisture The hypothesis under consideration were: 1. Chickpea plant can survive well under one month moisture stress as it stores enough water in its tissues. 2. There is considerable drought recovery in chickpea due to indeterminate plant growth habits. 3. Drought stress at pre-flowering stage is more detrimental to chickpea plant. The drought screening nursery must experience severe drought during the critical stages of plant growth. Therefore, such experiments regarding screening of germ plasm are needed to conduct under the control environment to avoid the risk of rainfall interruption during the imposition of moisture stress at the specific plant growth stages.

Materials and Methods

The experiment was conducted in Glasshouse at research site of Centre of Arid Zone Studies (CAZS), Natural Resources, University of Wales, Bangor, United Kingdom during 2007. Forty chickpea breeding lines (24 desi and 12 kabuli type) including four approved varieties viz. Paidar-91, Karak-1, Sheenghar-2000, and C-44 were included in this screening. All the germ plasm accessions were procured from various pulses research institutions of Pakistan. The experiment was laid out in Randomized Complete Block Design with three replications. The seeds of each line were first germinated in P12 plug Tray. Seven day-old seedlings were then transferred to Bench Top Raised Bed (BTRB) measuring 50 × 50 × 30 cm. Each BTRB comprised of four plants of different lines. The data were recorded from four plants in in each replication for each treatment. The glasshouse temperature was maintained at 18°C (8 h) and 15°C (16 h) during day and night respectively. The crop was maintained well and regularly irrigated up to pre-stress period. The moisture stress was imposed to the crop at pre-flowering stage for a period of 30 days (Yaqoob et al., 2011). After the stress was terminated, the crop had been regularly irrigated till harvesting. The crop observations were recorded on following parameters. Plant Wilting, Plant Wilting-II, Plant Recovery Response, and Grain Yield per plant (g).

The observation regarding first and second wilting were respectively recorded 20 and 30 days after commencing of moisture stress on 1-9 rating scale whereas plant recovery response was noted 10 days after re-watering to the crop. The grain yield was recorded in grams on per plant basis. Plant wilting and plant recovery were recorded as shown in Table 1.

Table 1. Plant wilting and plant recovery.

Plant Wilting rating		Plant Recovery Rating	
Score	Symptom of pant wilting	Score	Symptom of pant Recovery
1	Healthy plants	1	100% plant recovered
3	Slight foliar damage(11-20% on leave)	3	61-80% plant recovered
5	41-60% withering/drying in plant	5	41-60% plant recovered
7	61-80% withering/drying in plant	7	21-40% plant recovered
9	100% plant killing	9	No plant recovery

Statistical Analysis

The data acquired were subjected to analysis of variance based on RCBD using SPSS software version 12.0.

Results and Discussion

The results of analysis of variance regarding Wilting-I and Wilting-II, Plant Recovery and grain yield are given in Table 2 while means for these traits are given in Table 3. A study of Table 2 revealed highly significant variation for all the traits due to various chickpea lines/varieties.

Plant wilting-I

The analysis of variance regarding plant wilting-I showed highly significantly different among various chickpea lines/varieties (Table 2). The plant Wilting-I data recorded 20 days after imposing moisture stress ranged from 1 to 5 at 1-9 rating scale. There found variable trend of chickpea genotypes against moisture stress. The lines including SL-05-30 and SL-05-68 were found the best among all the genotypes and remained unaffected due to moisture stress by scoring 1. These lines were very closely followed by NCS950219, NCS05017, SL-03-11, SL-03-25, and C44 each having score of 1.67 (Table 3). The genotypes NCS9906, NCS05012, NCS05016, Karak-1, SL-03-14, SL-03-15, F-98-96C, F-97-112C, Sheenghar-2000 and F-97-168-C were also slightly affected at initial stage and their wilting score hardly reached to 2.00. There also found some lines which scored 3.00 or even low, these were NCS0501, SL-03-16, SL-03-7, SL-03-8, SL-03-16, SL-03-21, F-97-171C, Paaidar-91 and NCS05018. Out of forty, three genotypes namely, NCS9917, 90261, and NCS05010 were found to be more sensitive to moisture stress and showed more than 50% plant wilting recorded 20 days after moisture stress. The response of most of the lines indicated that chickpea crop easily escaped through moisture stress conditions up to 20 days. Among the lines, Kabuli types were found more sensitive to moisture stress as compared the desi type. The plant wilting in both the types could not exceed score 5. The highest wilting score (5) was found in kabuli type. Results revealed that chickpea genotypes were found sensitive to temperature and also remained type specific. The studies further showed that desi and kabuli types had variable response to abiotic stresses. The kabuli type chickpea were found more sensitive to high temperature and moisture stress than desi chickpea. Wang et al. (2006) had also found significant variation in desi and kabuli type chickpea in response to moisture and high temperature stress. This was due to the reason that kabuli types are adapted to Mediterranean region and therefore is more sensitive to high temperature and drought as compared to desi type (Leport et al., 2006). Matsui and Singh (2003) and Anbessa and Bejiga (2002) have also selected drought tolerance genotypes among chickpea accessions and reported reduced water loss from plant and extensive extraction of soil moisture as the factor of adaptation in drought tolerant genotypes.

Plant wilting-II

The data provided in Table 3 revealed that plant wilting-II had linearly increased in chickpea with increasing days to moisture stress. The genotypic trend of wilting-II has been directly proportional to the Wilting-I as well as to moisture stress period. Although, plant-wilting rate was quite divergent in different genotypes but their drought tolerance trend remained consistent in Wilting-I and Wilting-II. The wilting-II ranged from 2 to 7 (at 1-9 rating scale). The line SL-05-30 has again showed resistance to drought as it was slightly affected by drought after 30 days scoring 1.67. Some other desirable lines followed it were, NCS05019, NCS05017, SL-05-68 and C-44 each scored 2. These lines had also shown resistance to drought at Wilting-I. The lines Bittle-98, CMC211S, NCS9914, NCS9917, 90261, NCS05010, and NCS05014 were badly stroked by drought stress after 30 days with maximum wilting score of 7 by Bittle-98 (7.00). The performance of these lines was also not encouraging in Wilting-I.

Table 2. Mean squares of chickpea genotypes as affected by moisture stress.

Source of Variation	D.F	Plant Wilting-I 1-9 Scale	Plant Wilting-II 1-9 Scale	Plant Recovery 1-9 Scale	Grain yield Per plant (g)
Variation	39	4.289	10.045	14.757	75.823
Replications	2	0.896**	0.362**	9.923**	8.842**
Error	78	0.550	1.375	4.421	6.712
Total	120	-	-	-	-

Table 3. Plant wilting-I, Plant wilting-II, plant recovery and grain yield of various chickpea genotypes as under moisture stress.

Genotypes	Wilting-I	Witling-II	Grain yield plan^{-1}	Plant Recovery	Genotypes	Wilting-I	Witling-II	Grain yield plan^{-1}	Plant Recovery
SL-05-30	1 H	1.67 N	18.43A	3 F	NCS05019	2.67CD	4.67FG	4.93 STU	7.33 CD
C-44	1.67G	2 LM	12.47K	4.67EF	SL-03-16	2.67CD	4.67FG	6.23 PQRST	8.67 AB
NCS05017	1.67G	2 LM	17.79ABCDE	3 F	Paidar-91	3 CD	4.67FG	7.19 NOPQ	4.33 F
SL-05-68	1 H	2 LM	17.82 ABCD	5.33EF	NCS05013	3.33CD	4.67FG	9.88 M	9 A
NCS950219	1.67G	2 LM	18.33 AB	5.67EF	SL-03-01	2.67CD	4.67FG	12.22KL	4.33 FG
SL-03-11	1.67G	2.67 JKL	5 STU	7.33CD	F-98-96C	2 FG	4.67FG	14.41FGHJ	4.67 EF
NCS05018	2.33 EF	2.67 JKL	5.76 QRST	4 F	SL-03-15	2 FG	4.67FG	15.01FGHI	6 DE
F-97-168-C	2 FG	3 JK	6.22 PQRST	3 F	SL-03-7	2.67CD	5 EF	4.03 UV	8 AC
SL-03-25	1.67G	3.33 IJ	6.33 PQRST	4 F	NCS50204	4 B	5 EF	6.52 PQRS	8 AC
NCS05016	2 FG	3.33 IJ	6.58 PQRS	4.33F	NCS0502	4.67A	5 EF	6.56 PQRS	7.67 AC
Sheenghar-2000	2 FG	3.33 IJ	7.06 OPQ	4.67EF	SL-03-13	4 B	5.33DEF	6.87 PQR	9 A
SL-03-14	2 FG	3.33 IJ	15.19FGH	7.67AC	NCS05015	4.67A	5.67CDE	4.74 TUV	8.67 AB
Karak-1	2 FG	3.33 IJ	15.3 FG	4.33F	NCS0501	4 B	5.67CDE	8.85 MNO	9 A
NCS05012	2 FG	3.33 IJ	15.71 F	7.33CD	CMC211S	4 B	6 CDE	3.2 V	9 A
F-97-112C	2 FG	3.33 IJ	18.23ABC	3 F	90261	5 A	6 BCD	6.53 PQRS	9 A
SL-03-8	2.33EF	3.67 I	4.91 STU	4.67EF	NCS9917	5 A	6.33BC	4.01 UV	8.67 AB
F-97-171C	3 CD	4G HI	7.03PQR	9 A	NCS05010	5 A	6.33AB	6.00 PQRST	9 A
NCS05011	3.67BC	4.33 GH	4.03 UV	7.33CD	NCS05014	3 CD	6.67AB	5.04 STU	9 A
NCS9906	2F G	4.33 GH	7.58 P	6.33D	NCS9914	4.67A	6.67AB	7.59 NO	9 A
SL-03-21	2.33EF	4.33 GH	8.98MN	5 EF	Bittle-98	4.67A	7:00 AM	5.39 RSTU	8 AC
Mean	2.84	4.28	6.50	9.10	-	-	-	-	-
Range	1-5	1.67-7	3-9	3.2-18.43	-	-	-	-	-
S.E.	0.354	0.553	0.991	1.22	-	-	-	-	

The genotypic means have been arranged in their merit of ascending order in plant wilting-II.

The moisture stress had significant effect on number of flowers, fruiting nodes, pods, seeds and harvest index (Anyia et al., 2004). Kabuli types chickpea were found more sensitive to moisture stress than desi type in wilting-I and Wilting-II. The Desi type had higher yield than kabuli type chickpea (Leport et al., 2006). Leaf moisture retention index (LMRI) is an easily measurable physiological trait reflecting leaf turger maintenance under moisture stress and may be related to drought tolerance (Gupta and Sharma, 2006). Kabuli types have greater pod abortion than desi type (Leport et al., 2005). Wang et al. (2006) also reported that moisture stress was more damaging to kabuli type as compared to desi chickpea.

Plant Recovery Response

Re-sprouting and recovery of crop plant after termination of drought or any other biotic or abiotic stress depends upon the capability of genotype. The efficient genotypes usually have enough Leaf Water Potential (LWP) and also retain water in shoot tissues. The plant, ultimately utilizes the stored water, when it experienced moisture stress. The extent of plants survival under stress conditions and magnitude of their recovery after termination of stress depend upon their genetic potential. The plant recovery response in present investigations revealed that more than 50% lines could not properly recover and scored 7. While other lines though showed some recovery but could neither re-produce properly nor did they develop flowers and/or pod after revitalization. The lines NCS05017, SL-05-30 and F-97-112C and F-97-16C showed great recovery and scored 3 while most of the lines could not properly heal or recover slightly. Leport et al. (1999) reported that declining in photosynthesis in chickpea due to drought stress follows linear trend. Decreases in photosynthesis in cowpea under drought are largely attributed to effect of senescence with the increase of water (Quarrie and Jones, 1979). The net photosynthetic assimilation rate after drought termination is generally higher and it increases the dry matter accumulations in some cowpea genotypes (Anyia and Herzy, 2000). Berger et al. (1989) reported higher degree of biomass reduction in leaves and stem under rainfed conditions. They further suggested that physiological mechanism in addition to rapid phenology development had major role in chickpea adaptation to drought conditions. Yadav et al. (2005) have identified ICC4958 and FLIP87-59C as drought tolerant chickpea lines. The plant recovered greatly, at early pre-flowering stress as compared to pod formation stage. They also observed the higher yield of kabuli chickpea as compared to desi variety. The decrease in Leaf Water Potential (LWP) was faster in kabuki type chickpea as compared to desi type (Nayyar et al. 2006). Aslam et al. (2008) have also reported significant variation in morphological, physiological, phonological characters and yield and yield components in chickpea. The studies of Karadavut et al. (2010) also revealed significant character variability in fababean genotypes under different environments.

Grain yield

There exists highly significant variation in grain yield of various chickpea lines (Table 2). The grain yield ranged from 3.20 to 18.43 grams per plant. It was observed that the lines that were least affected by drought had produced higher yield, but this rule was not common in all the genotypes because, some of the lines having low first wilting score showed poor yield and conversely, there identified some other lines showing high wilting score and low yield. Lines SL-05-30, NCS950219 and F-97-112C with 18.43, 18.33 and 18.23 grams per plant produced the highest yield respectively. Another high yielding group including SL-05-68, NCS05017, NCS05012 and Karak-1 with grain yield of 17.82, 17.79, 15.71 and 15.30 grams per plant respectively followed the above lines. The lines SL-03-14, SL-03-15 and F-98-96C had also produced reasonable yield with 15.19 and 15.01 and 14.41 gram per plant respectively. The lines CMC 211S, SL-03-07 and NCS05015 with highest wilting and poor recovery score remained at the bottom and produced very low yield of only 3.24 and 4.74 gram pr plant respectively. It was also noted that the higher yields were mostly produced by the lines belong to desi type while the kabuli type produced lower yield under moisture stress conditions (Table 3). Similarly, data regarding plant wilting at 1-9 rating scale recorded at two stages during moisture stress had also showed that desi type chickpea possess more resistance to drought than kabuli type. It was interestingly noted that a few lines namely, NCS05016, SL-03-11, C44, NCS05018 and F-97-168C also produced poor yield despite low wilting and good plant recovery score. Mid-season drought had considerable reduction in plant activities and losses in biomass as well as in grain yield (Anyia and Herzy, 2000). Wang et al. (2006) exposed various chickpea lines at pre-flowering and pod formation stage under higher temperature and observed maximum yield damage by 59% due to higher temperature at pod

formation stage as compared to 34% yield reduction at pre-flowering stage. The results of Nayyar et al. (2006) revealed that 14 days water stress, to kabuli type chickpea exhibited 80% reduction in yield as compared with 64% reduction observed in desi chickpea and also noted that decrease in water leave potential was faster in kabuli as compared to desi. Singh et al. (1997) also confirmed that desi chickpea produces higher pods and yield than kabuli type. The chickpea lines with higher plant water status and high leaf water potential (LWP) produce higher number of seed per plant, seed/pod, harvest index and grain yield (Yadav et al., 2005). High temperature stress reduces the seed yield in desi as well as kabuli chickpea. They also have significant variation in various yield traits due to water stress. The lines having resistant to drought can be utilized in breeding programme for developing drought resistant lines. Desi types were found more tolerant to moisture stress coupled with high temperature as compared to kabuli type and ultimately produced higher yield. The pods abortion percentage was more sever in kabuli type chickpea as compared to Desi type under moisture stress and high temperature (Leport et al., 2006).

In present investigations, it has been observed that various chickpea lines had a quite divergent response to drought stress conditions. Out of 40 genotypes, ten had shown tolerance against drought prone conditions. These ten lines namely, SL-05-30, NCS950219, F-97-112-C, SL-05-68, NCS05017, NCS05012, Karak-1, SL-03-14, SL-03-15 and F-98-96-C remained superior all the time and gave higher yield of 18.43, 18.33, 18.23, 17.82, 17.79, 15.71, 15.30, 15.19, 15.01 and 14.41 grams per plant respectively. Similarly, plant recovery response revealed that more than 50% lines could not properly convalesce after termination of drought stress. This showed greater variability in chickpea lines in response to drought stress. The sensitivity of remaining 30 lines also suggests that moisture stress at pre-flowering is quite dangerous to chickpea plants. Kabuli type proved to be the more sensitive to moisture stress conditions as compared to desi type chickpea. These lines may therefore, be involved in screening/breeding for drought tolerance chickpea. In our present discussion, it has been observed that chickpea screening against drought environment is only possible under control environment. Moreover, among the various drought stresses at different crop growth stages, the stress at pre-flowering stage has been established to be the more fatal (Yaqoob et al., 2011). It is also suggested that chickpea lines should be given moisture stress at pre-flowering stage while screening against drought stress conditions.

Acknowledgements

The Principal Author Dr. Muhamamd Yaqoob thankfully acknowledges the financial support of Higher Education Commission of Pakistan for completing the above research project during post doctorate at UK.

References

Anyia, A. O. and H. Herzog. 2004. Genotypic variability in drought performance and recovery in cowpea under controlled environment. J. Agron. Crop Sci. 190:151-159.

Anbessa, Y. and G. Bejiga. 2002. Evaluation of Ethiopian chickpea landraces for tolerance to drought. Gen. Res. Crop Evol. 49(6):557-564.

Aslam, M. M., M. R. Ismail, M. Ashrafuzzaman, K. M. Shamsuzzaman and M. M. Islam. 2008. Evaluation of chickpea lines/mutants for high growth and yield attributes. Int. J. Agric. Biol. 10(5):493-498.

Berger, J. D., N. C. Turner, K. H. M. Siddique, E. J. Knights, R. B. Brinsmead, I. Mock, C. Edmondson and T. N. Khan. 2004. Genotypes by environment studies across Australia reveal the importance of chickpea (*Cicer arietinum* L.) Improvement. Aust. J. Agric. Res. 55:1071-1084.

Canci, H. and C. Tokar. 2009. Evaluation of yield criteria for drought and heat resistance in chickpea (*Cicer arietinum* L.). J. Agron. Crop Sci. 195(10):47-54.

Gupta, S. C. and S. N. Sharma. 2006. Leaf moisture retention index (LMRI): an easily measurable physiological characteristic for drought tolerance in chickpea. Ind. J. Pulses Res. 19(1):83-84.

Ismail, M. M., M. R. Ismail, M. Sharfuzzaman, K. M. Shamsuzzaman and M. M. Islam. 2008. Evaluation of chickpea lines/mutants for high growth and yield attributes. Int. J. Agric. Biol. 10(5):493-498.

Karadavut, U., C. Palta, Z. Kavurmaci and Y. Bolek. 2010. Some grain yield parameters of multi-environmental traits in faba bean (*Vicia faba*) genotypes. Int. J. Agric. Biol. 12(2):217-220.

Kashiwagi, J., L. Krishnamurthy, H. D. Upadhyaya, H. Krishna, S. Chandra, V. Vadez and R. Serraj. 2005. Genetic variability of drought-avoidance root traits in the mini-core germplasm collection of chickpea (*Cicer arietinum* L.). Euphytica 146:213-222.

Khan, A. S., Samiullah and S. Siddique. 2010. Genetic variability and correlation among seedling traits of wheat (*Triticum aestivum* L.) under water stress. Int. J. Agric. Biol. 12(2):247-250.

Leport, L., N. C. Turner, R. J. French, D. Tennant, B. D. Thomson and K. H. M. Siddique. 1999. Water relation, gas exchange and growth of cool-season grain legume in a Mediterranean-type environment. Eur. J. Agron. 9:295-303.

Leport, L., N. C. Turner, S. L. Davies and K. H. M. Siddique. 2006. Variation in pod production and abortion among chickpea cultivars under terminal drought. Europ. J. Agron. 24:236-246.

Lopez, F. B., Y. S. Chuhan and C. Johanson. 1997. Effect of timing of drought stress on leaf area development and canopy light interception of short duration pigeonpea. J. Agron. Crop Sci. 178:1-7.

Malik, S. R., A. Bakhsh, M. A. Asif, U. Iqbal and S. M. Iqbal. 2010. Assessment of Genetic variability and interrelationship among some agronomic traits in chickpea. Int. J. Agric. Biol. 12(1):81-85.

Matsui, T. and B. B. Singh. 2003. Root characteristics in cowpea related to drought tolerance at the seedling stage. Exp. Agri. 39:29-38.

MINFAL, 2009. Agriculture Statistics of Pakistan, Ministry of Food, Agriculture and Livestock, Government of Pakistan.

Nayyar, H., G. Kaur, S. Kumar and H. D Upadhyaya. 2007. Low temperature effects during seed filling on chickpea genotypes (*Cicer arietinm* L.): Probing mechanism affecting seed reserves and yield. J. Agro. Crop Sci. 2(4):1-8.

Shamsi, K. S. Kobraee and R. Haghparast. 2010. Drought stress mitigation using supplemental irrigation in rainfed chickpea (*Cicer arietinum* L.) varieties in Kermanshah, Iran. Afri. J. Biotec. 9(27):4197-4203.

Singh, K. B., M. Omar, M. C. Saxena and C. Johansen. 1997. Screening for drought resistance in spring chickpea in the Mediterranean region. J. Agron. Crop Sci. 178:227-235.

Wang, J., Y. T. Gan, F. Clarke and C. L. Mc Donald. 2006. Response of chickpea yield to high temperature stress during reproductive development. Crop Sci. 46:2171-2178.

Yadav, S. R., R. M. Yadav and C. Bhushan. 2005. Genotypic differences in physiological parameters and yield of chickpea (*Cicer arietinum* L.) under soil moisture stress conditions. Legume Res. 28:306-308.

Yaqoob, M, P. A. Hollington and J. Gorham. 2011. Development of field technique for mass screening of chickpea (*Cicer arietinum* L.) germ plasm under drought prone environments. Emir. J. Food Agric. 23(5):452-459.

Yaqoob, M, P. A. Hollington and J. Gorham. 2012. Shoot, root and flowering time studies in chickpea (*Cicer arietinum* L.) under two moisture regimes. Emir. J. Food Agric. 24(1):73-78.

Emir. J. Food Agric. 2013. 25 (2): 124-131
doi: 10.9755/ejfa.v25i2.11660
http://www.ejfa.info/

PLANT SCIENCE

A novel and efficient protocol for the isolation of genomic DNA from mulberry (*Morus* L.)

H. Jingade Anuradha[1], Kunjupillai Vijayan[2*], Chirakara V. Nair[1] and A. Manjula[1]

[1]*Central Sericultural Germplasm Resources Centre, Thally Road, Hosur, Tamil Nadu-635109, India*
[2]*Research Coordination Section, Central Silk Board, BTM Layout, Madiwala, Bangalore- 560068, India*

Abstract

The purity of DNA is one of the major factors affecting the success of Genomic studies. Nucleic acid isolation from polyphenol rich plants fail to produce good quality DNA or RNA as polyphenols adhere and interfere with DNA during isolation. An improvised, simple and inexpensive protocol has been developed for extracting genomic DNA from Mulberry (Morus spp.). The purity of the DNA as revealed by the ratios of absorbance at 260/280 nm (A 260/280) and 260/230 nm (A 260/230) was closer to 2.0. Genomic DNA analyzed for analytical applications like restriction digestion and PCR amplification with molecular markers viz., Inter Simple Sequence Repeats (ISSR), Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR) primers further confirmed the purity of the DNA. A modified method of silver staining was employed for the resolution of SSR amplified products. Physiologically mature leaf was found more suitable for getting quality DNA in mulberry.

Key words: Mulberry, DNA, ISSR, RAPD, SSR, Silver staining

Introduction

Extraction of quality DNA is a major challenge for molecular biologists dealing with tree plants. Many contaminating agents like, higher contents of polyphenolic compounds, resins, latex, and polysaccharides, tannins present in the cell as secondary metabolites usually co precipitate with DNA and interfere with the activity of the DNA polymerase enzyme (Pandey et al., 1996). Cell lysis process followed by the polyphenols oxidation and co-precipitation causes browning of the DNA (Varma et al., 2007). Viscous DNA samples have been obtained as a result of the co-precipitation of the gelling polysaccharide making the samples viscous and hamper the proper loading of the samples on the gel for electrophoresis (Diadema et al., 2003). The procedures for the extraction of plant DNA are modified continuously to get quality DNA (Cheng et al., 1997; Kobayashi et al., 1999; Karakousis and Langridge, 2003; Vijayan, 2004). Mulberry (*Morus* spp.) is a perennial tree having high economic value and is the sole food plant for the silkworm, *Bombyx mori* L. (Lepidoptera). Leaves of mulberry contain large amount of polysaccharides, polyphenols, and many sticky and resinous materials, which are not fully characterized. Mulberry leaf extract contains 44.82% polyphenols (Chan et al., 2009). Leaves have comparatively high values of phenolic compounds which ranged from 8.33 to 11.79 mmol/100 g of dry weight of leaves in three species of mulberry *Morus nigra, Morus alba* and *Mours laevigata* (Memon et al., 2010). Attempts to extract DNA from leaf samples using many of the existing protocols (Cheng et al., 1997; Kobayashi et al., 1999; Karakousis and Langridge, 2003; Vijayan, 2004) have resulted in isolation of DNA with limited success. In most cases, the DNA extracted has not amplified successfully with Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) primers. Molecular work in mulberry with microsatellite primers (SSR) is very much limited. The setback experienced in Genomic research in mulberry can be attributed to the poor quality DNA. To overcome this problem, commercial DNA extraction kits (Qiagen, Valencia, CA, USA; Amersham Life science, England) are used to extract Polymerase Chain Reaction (PCR) grade DNA from mulberry leaves (Vijayan et al., 2006; Awasthi et al., 2004; Chatterjee et al., 2004).

Received 30 March 2012; Revised 07 June 2012; Accepted 26 June 2012; Published Online 28 November 2012

*Corresponding Author

H. Jingade Anuradha
Scientist C, Central Sericultural Germplasm Resources Centre, Central Silk Board, Thally Road, Hosur-635 109, Tamil Nadu, India

Email: anujnh@yahoo.co.in

However usage of these commercial kits for routine extraction is economically difficult for large-scale genomic applications. In the present study, we report a simple, rapid and efficient method yielding appreciable levels of high quality DNA suitable for molecular studies including restriction digestion and PCR amplifications. PCR was carried out using molecular markers like RAPD, ISSR and SSR primers. A protocol of silver staining the Polyacrylamide Gel Electrophoresis (PAGE) gel for microsatellite analysis in mulberry is also established in the present study.

Materials and Methods

Collection of plant material

Five mulberry species namely *M. alba, M. rotundiloba, M. serrata, M. laevigata* and *M. nigra* grown in the germplasm of Central Sericultural Germplasm Resources Center (CSGRC), Hosur, Tamil Nadu, India were selected for the study. These plants were propagated through stem cuttings to maintain their genetic identity. Fresh leaf samples were collected from 90-day-old (after pruning) primary branches of these plants.

Isolation of genomic DNA

Protocol: The modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1990) was followed in the present study. Sorvall RC-5C High-speed centrifuge was used for the extraction of the mulberry DNA.

Steps for the extraction of Mulberry DNA

1. 100 mg of the leaf lamina was homogenized in a pre-cooled mortar and pestle, adding approximately 15 ml of liquid nitrogen
2. The leaf powder was transferred to 15 ml Oakridge tube
3. Added 600 µl of preheated (60^{o}C) extraction buffer containing Sodium chloride (NaCl), Polyvinyl pyrrolidone (PVP), Cetyltrimethyl ammonium bromide (CTAB), L-ascorbic acid, Diethyldithocarbamic acid, Sodium metabisulfite, Sodium dodecyl sulfate (SDS), Tris-EDTA (TE) in the concentrations as detailed above.
4. Mixed the leaf powder in the extraction buffer thoroughly by vortexing
5. Added 33.3 µl β-mercaptoethanol and incubated at 60°C in a shaking water bath for ten minutes.
6. Centrifuged at 20000g for five minutes, and transferred the supernatant to a new 1.5 ml Eppendorf tube.
7. Added 600 µl chloroform: isoamyl alcohol in the ratio of 24:1 and mixed thoroughly for three minutes by vortexing to form an emulsion. Centrifuged at 20000g for five minutes.
8. Transferred the aqueous phase to a new1.5 ml Eppendorf tube.
9. Added 500 µl of ice cold 100% isopropanol and briefly vortexed the tube and kept on ice for ten minutes. Centrifuged at 20000g for two minutes and pipetted out the supernatant carefully into a waste jar without disturbing the DNA at the bottom of the tube.
10. Dried the DNA in SpeedVac Vaccum Concentrator and dissolved in 100 µl of Tris-EDTA (TE) buffer containing 0.2 µl of RNAse A and vortexed to enable uniform mixing.
11. Placed the DNA solution in a 37°C heat block and incubated for ten minutes.
12. Added 50 µl of 7.5M Ammonium Acetate and 200 µl 95% ethanol kept on ice for ten minutes and centrifuged at 20000g for five minutes.
13. Decanted the supernatant carefully and washed the DNA with 500 µl 95% ethanol and centrifuged at 12000g for five minutes.
14. Pipetted out the ethanol and repeated the washing step as in step 13.
15. Dried the DNA in the SpeedVac Vacuum Concentrator at 45°C (low heat setting) for ten minutes.
16. Dissolved the DNA in 50 µl of 10 mM Tris (pH 8.0). Tris is used instead of TE, as the EDTA can inhibit enzymatic reactions including PCR.
17. Stored at -20°C till further use.

Table 1. Buffers and solutions used for the present protocol.

Buffers/Solutions	Contents
Sodium chloride	0.98g, 1.4M
Polyvinyl Pyrrolidone (PVP) (40 kD)	0.24g, 2.0%
Cetyl Trimethyl Ammonium Bromide (CTAB)	0.24g, 2.0%
L-ascorbic acid	0.01g, 5 mM
Diethyldithocarbamic acid (DIECA)	0.008g, 4 mM
Sodium Meta Bisulfate	0.12g, 1.0%
Sodium Dodecyl Sulfite (SDS)	0.06g, 0.5%
Tris	2.4 ml, 200 mM, 1 M, pH 8.0
Ethylene Diamine Tetra Aceticacid (EDTA) Disodium salt	480 µl, 20 mM, 0.5 M, pH 8.0
Milli Q Water	8.5 ml, (18.2 MΩ)
Liquid Nitrogen	For quick freezing the plant material

Analysis of the genomic DNA

The resultant genomic DNA was electrophoresed for qualitative analysis on 0.8%. agarose gel containing ethidium bromide at a final concentration of 0.5 µg/ml. The DNA yield per gram of leaf tissue was measured by using a UV spectrophotometer (Shimadzu, Japan) at 260 nm. DNA purity was determined by calculating the absorbance $A_{260/280}$ ratio. Polyphenol contamination was assessed by calculating the absorbance ratio $A_{260/230.}$ The purity of DNA was further analyzed for its analytical applications like Polymerase chain reaction amplifications and restriction digestion.

PCR amplification of RAPD and ISSR primers

PCR amplification of the DNA with RAPD (Random Amplified Polymorphic DNA) primer was carried out as described by Chatterjee et al. (2004) on an MJ Research Thermal-Cycler, PTC-200 using 20 µl of reaction mixture containing 1 X PCR Buffer (MBI Fermentas) 200 µM each of dGTP, dATP, dCTP and dTTP; 2.0 µM $Mgcl_2$; 100pM primer; 10 ng genomic DNA and 1 U Taq polymerase. The PCR schedule followed was 93°C for 2 min followed by 35 cycles of 93°C for 1 min, 36°C for 1 min, 72° C for 2 min and a final incubation at 72°C for 15 min. The ISSR (Inter Simple Sequence Repeat) amplification was carried out as per Vijayan and Chatterjee (2003) using 20 µl reaction mixture containing 2.0 µl of 10 X PCR buffer (750 mM Tric-HCl pH 8.8; 200 mM $(NH_4)_2SO_4$; 0.1% Tween-20), 0.2 mM dNTP, 2 mM $MgCl_2$; 200nM Primer; 50 ng genomic DNA and 1 U Taq DNA polymerase (MBI Fermentas Inc, Hanover, MD-21076, USA). The PCR schedule included an initial cycle at 94°C for 2 min followed by 35 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 2 min and a final extension of 10 min at 72°C. The PCR products were separated in 1.5% agarose gel in 1 X Tris Boric Acid buffer (TBE) containing 0.5 µg/ml Ethidium Bromide as stain.

PCR amplification of microsatellite primers (SSR)

Five pairs of simple sequence repeat primers developed by Aggarwal et al., (2004) were used for the study. The PCR amplification was carried out in 20 µl reaction buffer containg 10 ng template DNA, 1 pmol of each primer, 2 mM of $Mgcl_2$, 100 µM of dNTPs, 1 X PCR buffer, 1 U DNA polymerase (Aggarwal et al., 2004) on an M.J Research Thermal-Cycler, PTC-200 (MJ Research Inc. Massachusetts, USA). The PCR schedule included initial denaturation at 94°C for 10 min followed by 35 cycles of 94°C for 1 min denaturation, primer-specific annealing temperature for 1 min and 72°C for 1.5 min extension followed by the final extension step of 72°C for 5 min.

Resolution of PCR-SSR amplification

The microsatellite amplicons was resolved in Polyacrylamide gels. The Polyacrylamide gel was prepared in a Pharmacia LKB Macrophor sequencing gel system. The gel casting plates were cleaned thoroughly with liquid soap and water, wiped with double distilled alcohol and allowed to dry. The smaller gel plate (33.3 x 39.4 cm) was treated with Bind Silane containing 20 µl of γ-methacryloxypropyl-trimethoxysilane (PlusOne Bind Silane, Amersham Pharmacia Biotech) in 1.5 ml of 10% acetic acid and 5ml of double distilled alcohol. The plate was kept for drying for 15 minutes. The larger plate (33.3 x 41.9 cm) was similarly cleaned with liquid soap and water and subsequently dried with alcohol. This plate was treated with 1ml of 2% Dimethyldichlorosilane in octamethyl cyclo- octasilane (Plus One Repel Silane, Amersham Pharmacia Biotech). This plate was also allowed to dry for 15 minutes. After proper drying, the gel plates were assembled using 0.75 mm spacer and clips. 6% polyacrylamide gel was prepared by dissolving 32.5g Urea, 4.25g acrylamide and 0.225g N, N'-methylene bisacrylamide in 75 ml 1X TBE buffer (89 mM Tris-base; 89 mM boric acid; 2 M EDTA). The solution was filtered through Whatman-1 filter paper. Just before casting the gel 600 µl of 10% freshly prepared Ammonium persulfate and 20 µl of Tetramethylethylenediamine (TEMED) were added to the solution. The gel solution was poured into the gel assembly slowly without trapping any air bubbles. The gel was allowed to polymerise for two to four hours. The gel was pre-run at 600 V for one hour at 45°C before loading the sample. The sample was prepared by mixing three parts of PCR product with one part of loading dye containing 95% Formamide (v/V), 10 mM NaOH, 20 mM EDTA, 0.05% (w/v) Bromophenol blue and 0.05% (w/v) Xylenecyanol. The sample was denatured, by heating at 95ºC for 5 minutes and keeping on ice immediately. About 6 µl of the sample was loaded to the gel and run it at 600 V for five hours at 45°C. Phage ØX174 DNA digested with *Hinf* I (MBI, Fermentas) was used as size marker.

Silver Staining of Polyacrylamide Gel

The staining of the gel was carried out using a modified protocol of Sanguinetti et al. (1994). Following electrophoresis, the gel bound to the gel

plate was fixed in 10% (v/v) Ethanol along with 0.5 ml/100ml acetic acid for 45 minutes. The gel was then quickly treated with 2% citric acid for 90 seconds. The gel was subsequently rinsed twice with double distilled water (ddH_2O) and transferred to 0.2% (w/v) Silver nitrate solution for 30 minutes with occasional shaking. The gel after a quick rinsing in double distilled H_2O was transferred to a solution containing 3.0% (w/v) Sodium hydroxide and 7.5 ml/L 37% formaldehyde. After appropriate development of the bands, the gel was transferred to Stop solution containing 1.5% (w/v) EDTA. The gel was photographed under white fluorescent light and data were scored directly from the gel on the basis of presence or absence of markers.

Endonuclease digestion

The suitability of the DNA for hybridization-based techniques was tested by subjecting 10 µg of genomic DNA with 30 U of EcoRI (Boehringer Mannheim, Germany) in the recommended buffer and incubated at 37ºC for 6 hours. The digestion mixture was subjected to electrophoresis in 1.0% agarose gel and observed under Ultra Violet (UV) transilluminator (Amersham Pharmacia Biotech).

Results and Discussion

The method described here could isolate good amount of high quality DNA, which was evident from spectrophotometric analysis**,** PCR amplification and endonuclease digestion of the DNA. The spectophotometric analysis at $A_{260/\ 280}$ gave a ratio ranging from 1.74 ± 0.14 to 1.95 ± 0.03, which indicates very little contamination from proteins, as proteins absorb at A_{280}. Likewise the $A_{260/230}$ ratio of near 2.0 indicates the absence of contaminants like carbohydrates, peptides and phenolic compounds in the DNA. Electrophoresis on agarose gel further revealed the absence of shearing in the DNA (Figure 1). The PCR amplification with ISSR, RAPD and SSR primers (Figures 2 to 4) and the complete digestion of the DNA with restriction endonuclease (Figure 5) further confirmed the absence of contaminants interfering PCR and restriction endonuclease digestion. It is a known fact that contaminants like polysaccharides and polyphenols are, in general, difficult to be removed from the DNA as they often adsorb and co-precipitate with the DNA (Murray and Thompson, 1980) and interfere with PCR and restriction endonuclease digestion. Polysaccharides also interfere with several biological enzymes such as polymerases, ligases and restriction endonucleases (Shioda and Murakami, 1987; Richards, 1988).

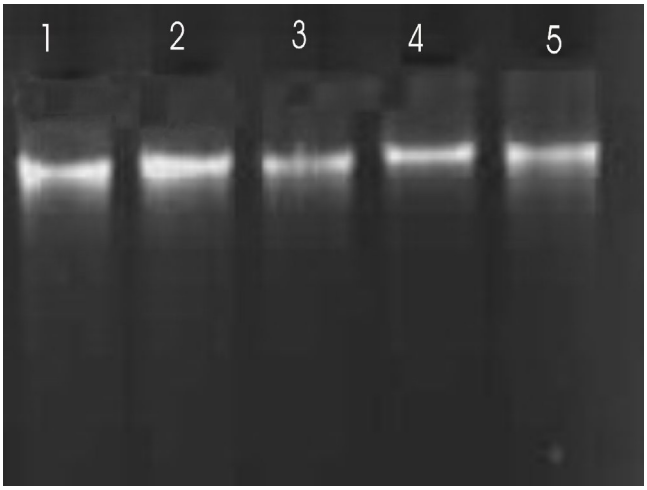


Figure 1. Genomic DNA extracted from five mulberry species. 1. ***M. alba***, 2. ***M. rotundiloba***, 3. ***M. serrata***, 4. ***M. laevigata***, 5. ***M. nigra***.

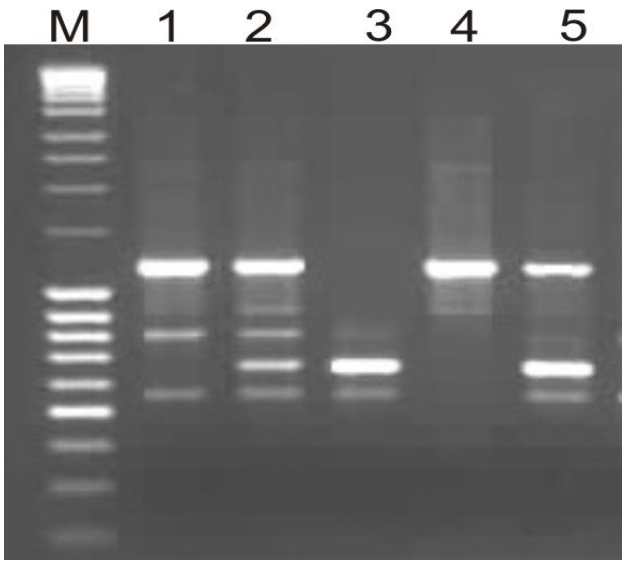


Figure 2. PCR amplification of the DNA with ISSR primer UBC-812. M-marker. 1. *M. alba*, 2. *M. rotundiloba*, 3. *M. serrata*, 4. *M. laevigata*, 5. *M. nigra.*

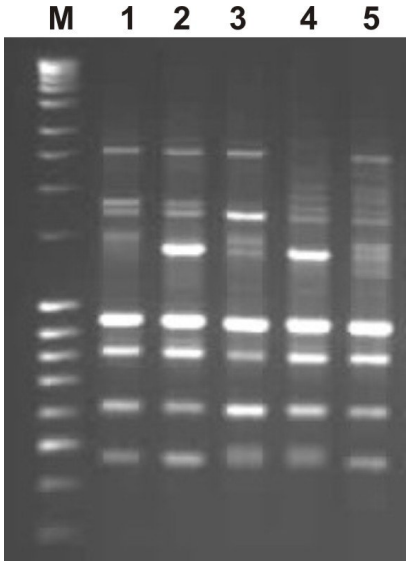


Figure 3. PCR amplification of the DNA with RAPD primer OPW-4. M-marker. 1. *M. alba*, 2. *M. rotundiloba*, 3. *M. serrata*, 4. *M. laevigata*, 5. *M. nigra*.

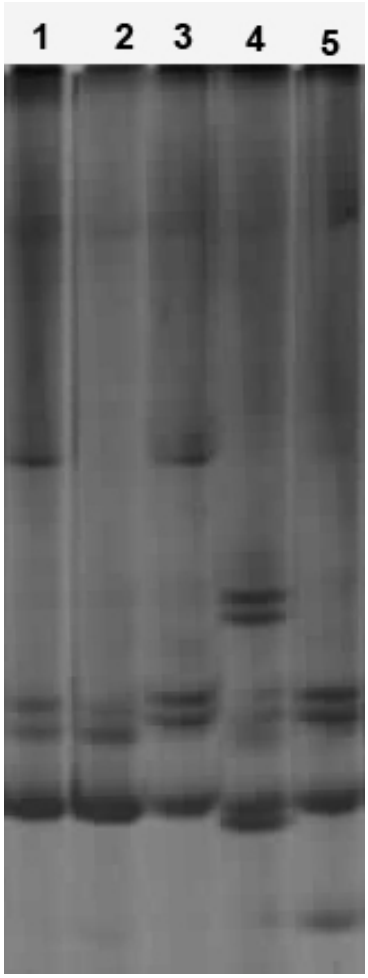


Figure 4. PCR amplification of the DNA with SSR primer MUL-5 and the gel was stained with silver nitrate. 1. *M. alba*, 2. *M. rotundiloba*, 3. *M. serrata*, 4. M. *laevigata*, 5. *M. nigra*

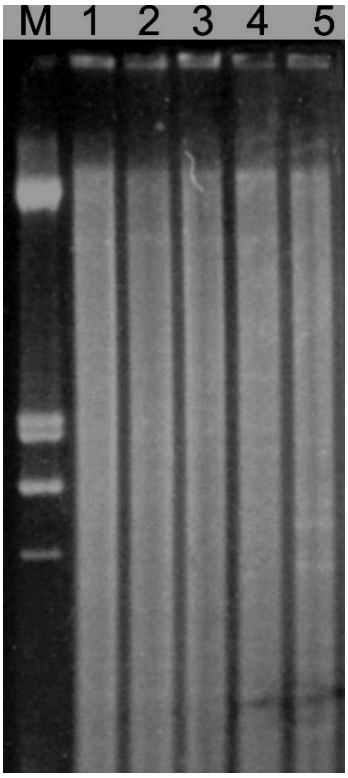


Figure 5. Endonuclease restriction digestion of the DNA with EcoRI. M. Marker. 1. *M. alba*, 2. *M. rotundiloba*, 3. *M. serrata*, 4. *M. laevigata*, 5. *M. nigra*.

The major modifications in the present study from the standard Cetyl Trimethyl Ammonium Bromide (CTAB) protocol (Doyle and Doyle, 1990) are the addition of polyvinyl pyrrolidone (PVP), L-ascorbic acid, diethyldithocarbamic acid (DIECA), sodium metabisulfite and sodium dodecyl sulfate in the extraction buffer. DIECA is a phenoloxidase inhibitor and it helps reduce brown colouring due to oxidation of polyphenols to quinons. Addition of L-ascorbic acid prevents oxidation of phenolic compounds and adsorption by DNA molecules. Similarly, PVP forms complex compounds by hydrogen bonding with phenolic compounds and co- precipitates along with cell debris thus preventing polyphenols from interacting with DNA. When the extract is centrifuged in the presence of chloroform, the PVP complexes accumulate at the interface between the organic and the aqueous phases (Kim et al., 1997; Barwell et al., 1998). CTAB is a cationic detergent, which solubilizes membranes and also binds to fructans and other polysaccharides to form complexes that are removed during subsequent chloroform extraction. The time taken for the extraction of DNA is very much less compared to the other protocols and the entire procedure could be completed within 2 hours. This method is a one-step extraction procedure, which includes the RNAase treatment for the removal of RNA. In this study the suitability of physiologically mature leaf (7th-9th leaf from the tip) over young leaf was observed. When samples from very young leaves were used**,** the DNA yield and quality was very poor. Hence, it is found better to use physiologically mature mulberry leaf for DNA extraction. This finding of low and poor quality of DNA from immature leaves is in complete agreement with the reports of Small et al. (2004) that in certain plants mature leaves are more suitable for DNA extraction. Another point needs mentioning here is the low ratio of $A_{260/280}$ observed in *M. serrata.* It is a known fact that the leaves of *M. serrata* are much leathery, feathery and thicker than the leaves of other species (Tikader and Dandin, 2001). However, the complete digestion of the DNA with restriction endocnuclease enzyme indicates that DNA is devoid of contaminants interfering digestion. Although silver staining was initially used for detection of proteins separated by Polyacrylamide gel electrophoresis (Merril et al., 1981) later its use in detection of nucleic acid on Polyacrylamide gel was realized (Sanguinetti et al., 1994). Using different modifications to the original protocols Bassam et al. (1991) enhanced the sensitivity of the staining to 1pg/mm² by different modifications such as inclusion of Sodium thiosulphate, pretreatment with Potassium dichromate and nitric acid. Sanguinetti et al. (1994) on the other hand used Sodium hydroxide to establish an alkaline environment for the silver ion to reduce into metallic silver in the presence of formaldehyde. However, all these protocols were developed for native or denaturing gels detached from the gel plates after electrophoresis. Since, in most of the SSR analysis, electrophoresis in long and thin gel is required for better separation of the alleles, separation of the gel from the glass plate is found difficult. In this report, we have developed a method for staining the thin Polyacrylamide gel still attached to the glass plate. In this protocol we have included an additional step of pretreatment of the gel plate with citric acid, which has enhanced the band visibility, by reducing the background staining considerably. Similarly, the use of higher formaldehyde concentration than the one used by Sanguinetti et al. (1994) resulted in darker bands with good contrast. Further, this modified staining protocol for large sequencing gels bound to glass has many advantages like it requires small amount of the sample, a large number of samples could be analyzed in a single run and most of the staining reagents can be reused without loss of quality.

Conclusion

The present protocol is suitable for isolating high quality DNA from leaves of tree plants containing higher amounts of polysaccharides, polyphenols, resins and other viscous contaminants. The successful endonuclease digestion and the amplifications of the different molecular markers confirm the purity of the DNA. This protocol is simple, rapid, inexpensive and easily amenable to other species.

References

Aggarwal, R. K., D. Udaykumar, P. S. Hendre, A. Sarkar and L. I. Singh. 2004. Isolation and characterization of six novel microsatellite markers for mulberry (*Morus indica*). Mol. Ecol. Notes 4:477-479.

Awasthi, A. K., G. M. Nagaraja, G. V. Naik, K. Sriramana, K. Thangavelu and J. Nagaraju. 2004. Genetic diversity in mulberry (genus *Morus*) as revealed by RAPD and ISSR marker assays. BMC genetics, 5, 1 (available from: http://www.biomedcentral.com/1471-2156/5/1).

Barwell, P., A. N. Blanchard, J. A. Bryant, N. Smirnoff and A. F. Weir. 1998. Isolation of DNA from highly mucilaginous succulent plant *Sedum telephium*, Plant Mol. Biol. Report 16:133-138.

Bassam, B. J., G. Caetano-Anolles and P. M. Gresshoff. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem. 196:80-243.

Chan, K. C., H. H. Ho, C. N. Huang, M. C. Lin, H. M. Chen and C. J. Wang. 2009. Mulberry leaf extract inhibits vascular smooth muscle cell migration involving a block of small GTPase and Akt/NF-kB signals. J. Agric. Food Chem. 57(19):9147-9153.

Chatterjee, S. N., G. M. Nagaraja, P. P. Srivastava and G. Naik. 2004. Morphological and molecular variation of *Morus laevigata* in India. Genetica 121:133-143.

Cheng, P. S., S. K. Brown and N. P. Weeden. 1997. A DNA extraction protocol from various tissues in woody species. Horticultural Science 32:921-922.

Doyle, J. J. and J. L. Doyle. 1990. Isolation of plant DNA from fresh tissue. Focus 12:13-15.

Diadema, K., A. Baumel, M. Lebris and L. Affre. 2003. Genomic DNA isolation and amplification from callus culture in succulent plants, *Carpobrotus* species (Aizoaceae). Plant Mol. Biol. Report 21:173a-173e.

Karakousis, A. and P. Langridge. 2003. A high throughput plant DNA extraction method for marker analysis. Plant Mol. Biol. Report. 21:95a-95f.

Kim, C. S., C. H. Lee, J. S. Shin, Y. S. Chung and J. E. Hyung. 1997. A simple and rapid method of isolation of high quality genomic DNA from fruit trees and conifers using PVP. Nucleic Acid Res. 27:66-58.

Kobayashi, N., K. Tamura and T. Aotsuka. 1999. PCR error and molecular population genetics. Biochem. Genet. 3:318-321.

Memon, A. A., N. Memon, L. D. L. Luthria, M. L. Bhanger and A. A. Pitafi. 2010. Phenolic acids profiling and antioxidant potential of mulberry (*Morus laeviagata*., *Morus nigra* L., *Morus alba* L.) leaves and fruits grown in Pakistan. Pol. J. Food Nutr. Sci. 60(1):25-32.

Merril, C. R., D. Goldman, S. A. Sedman and M. H. Ebert. 1981. Ultrasensitive stain for protein in polyacrylamide gel shows regional variation in cerebrospinal fluid proteins. Science 211:1437-1438.

Murray, M. G. and W. F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res. 8:4321-4325.

Pandey, R. N., R. P. Adams and L. E. Flournoy. 1996. Inhibition of random amplified polymorphic DNAs (RAPDs) by plant polysaccharides. Plant Mol. Biol. Report 14:17-22.

Richards, E. 1988. Preparation of genomic DNA from plant tissue. In: F. M. Ausubel, R. E. Kingston, D. D. Moore, J. A. Smith, J. G. Seidman and K. Struhl (Eds.). pp.2.3.2-2.3.3. Current Protocols in Molecular Biology. Greene Publishing Associates and Wiley-Interscience, New York.

Sanguinetti, C. J., E. D. Neto and A. J. G. Simpson. 1994. Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. Bio Techniques 16:915-919.

Shioda, M. and K. Marakami-Muofushi. 1987. Selective inhibition of DNA polymerase by a

polysaccharide purified from slime of *Physarum polycephalum*. Biochem. Biophys. Res. Commun. 146:61-66.

Small, R. L., R. C. Cronn and J. F. Wendel. 2004. The use of nuclear genes for phylogeny reconstruction in plants. Aust. Syst. Bot. 17:145-170.

Tikader, A., S. B. Dandin. 2001. Breeding behaviour of some wild mulberry. Indian Silk 40:9-10.

Varma, A., H. Padh and N. Shrivastava. 2007. Plant genomic DNA isolation: an art or a science. Biotechnol. J. 2:386-392.

Vijayan, K. 2004. Genetic relationships of Japanese and Indian mulberry (*Morus* spp.) genotypes revealed by DNA fingerprinting. Plant Syst. Evol. 243:221-232.

Vijayan, K. and S. N. Chatterjee. 2003. ISSR profiling of Indian cultivars of mulberry (*Morus* spp.) and its relevance to breeding programs. Euphytica 131:53-63

Vijayan, K., C. V. Nair and S. N. Chatterjee. 2006. Molecular characterization of mulberry genetic resources indigenous to India. Genet. Resour. Crop Ev. 52:77-86.

Emir. J. Food Agric. 2013. 25 (2): 132-137
doi: 10.9755/ejfa.v25i2.6084
http://www.ejfa.info/

PLANT SCIENCE

Heterosis and combining ability in a diallel among elite inbred lines of maize (*Zea mays* L.)

Mohammad Amiruzzaman, Md. Amirul Islam, Lutful Hasan, Monjurul Kadir and Md. Motiar Rohman*

Plant Breeding Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh

Abstract

Heterosis and combining ability was studied for grain yield, days to tasseling, days to silking, plant height and ear height in a diallel cross involving seven elite maize inbred lines. Variance due to GCA and SCA were highly significant for the characters studied, indicating both additive and non-additive type of gene action were important for controlling the traits. Predominance of non-additive gene action was observed for all the traits. Standard heterosis for grain yield ranged from -17.60 to 9.71%. For other traits, desirable heterosis varied from -0.10 to -4.42%; -0.03 to -4.20%; -2.44 to -42.11% and -1.33 to -21.87% for days to tasseling, days to silking, plant height and ear height, respectively. Parent Q7 was the best general combiner for higher grain yield coupled with dwarfness, and Q1 was also good general combiner for grain yield and lateness in maturity. For other traits, parent Q2, Q3 and Q4 were found suitable both for days to tasseling and silking and Q4, Q5 and Q7 for both plant and ear height showing desirable significant negative GCA effects and simultaneously possessed desirable high mean values, indicating that per se performance of the parents could prove as an useful index for combining ability. Additive × additive, additive × dominance and dominance × dominance gene interactions were involved in deriving good specific cross for yield. The cross combinations Q1 × Q7, Q2 × Q3, Q4 × Q6 and Q6 × Q7 possessing significant desirable SCA effects and high heterotic values might be used for obtaining high yielding hybrids.

Key words: Heterosis, Plant breeding, Combining ability, Maize, Quantitative breeding

Introduction

Exploitation of hybrid vigor and selection of parents based on combining ability has been used as an important breeding approach in crop improvement. Selection of parents on the basis of *per se* performance with good GCA effect is the high approach to assess the nature of gene action involved in the inheritance of character (Vasal, 1998). Combining ability analysis is one of the powerful tools in identifying the better combiners which may be hybridized to exploit heterosis and to select better crosses for direct use or further breeding work (Nigussie and Zelleke, 2001). Information on the heterotic patterns and combining ability among maize germplasm is essential in maximizing the effectiveness of hybrid development (Beck et al., 1990). Heterosis and combining ability is prerequisite for developing good economically viable hybrid variety in maize. Breeder's objectives are to select hybrids on the basis of expected level of heterosis as well as specific combining ability. The present investigation is therefore, aimed to know the gene action for yield and other important traits in maize inbred lines and to explore heterotic hybrid combinations.

Materials and Methods

Seven maize inbred lines viz. CML 487, CLG 1837, CML 480 and CML 223 originated from CIMMYT, Mexico; Ki 32 and Ki 42 from Kasrtsart Univ., Thailand and Tzi 24 from IITA, Nigeria designated as Q_1, Q_3, Q_6, Q_7, Q_2, Q_4, and Q_5 were crossed in a diallel fashion excluding reciprocals in the kharif II (rainy) season of 2005-06 with polyethylene sheed at the research farm of Bangladesh Agricultural Research Institute, Gazipur. In the following rabi (winter) season 2006-07, all the F_1 hybrids, their respective parents along with a commercial check, Pacific 11 were grown in the same farm following alpha lattice design (Patterson et al., 1978) with three replications. Each plot comprised two rows of 5 m

Received 03 January 2011; Revised 10 May 2011; Accepted 26 May 2011; Published Online 28 November 2012

*Corresponding Author

Md. Motiar Rohman
Plant Breeding Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh

Email: motiar_1@yahoo.com

long. Row to row and plant to plant spacing was 75 cm and 20 cm, respectively. One healthy seedling per hill was kept after proper thinning at two weeks after germination. Fertilizers were applied @ 250, 120, 120, 40 and 5 kg/ha of N, P_2O_5, K_2O, S and Zn, respectively. Standard agronomic practices were followed and plant protection measures were taken when required to ensure normal growth and development of the plants. Ten randomly selected competitive plants (5 from each row in a plot of each genotype in each replication) excluding any plant surrounding by a missing hill and border plants were used for recoding observations on grain yield (ton/ha), days to tasseling, days to silking, plant height (cm) and ear height (cm). Standard heteross was estimated and tested according to Singh and Singh (1994). Combining ability analysis was carried out following Model I Method 2 described by Griffing (1956) using CropStat (2007) software program. The mean squares for GCA and SCA were tested against their respective error variances derived from ANOVA reduced to mean level.

Results and Discussion

The analysis of variance showed that genotypes differed significantly for all the characters (Table 1). Analysis of variance for combining ability revealed that variance due to GCA and SCA effects were significant, indicates importance of additive as well as non-additive type of gene action. The results agree with the findings of several researchers (Mathur and Bhatnagar, 1995; Nass et al., 2000; Rokadia and Kaushik, 2005) who reported the importance of both additive and non-additive gene action in maize.

General combining ability variance (δ^2g) indicates additive (additive and additive × additive epistasis), while specific combining ability variance (δ^2s) indicates non-additive (dominance and additive × dominance, dominance × dominance) genetic variation (Griffing, 1956; Baker, 1978). So, the significant estimates of GCA and SCA variances suggest that both additive and non-additive gene actions were involved for all the characters.

The additive (σ^2A) and non-additive (σ^2D) genetic variance were estimated from GCA and SCA variances. Comparatively higher estimates of non-additive genetic variance over additive genetic variance were observed for the characters studied. Grain yield was predominantly controlled by non-additive gene action. This is corroborated with the earlier findings of Khotyleva et al. (1986) who reported that grain yield was more influenced by dominant than additive gene effects. Later on similar results were also obtained by Mathur and Bhatnagar (1995) and Zelleke (2000). Predominance of non-additive gene action for grain yield in maize was also reported by Dass et al. (1997). It could be concluded that the improvement of the characters with greater non-additive genetic component could be contemplated for the exploitation of heterosis, and with bi-parental mating.

General combining ability (GCA) effects

The estimates of GCA effects showed that none of the parent was general combiner for all the traits in desired direction (Table 2). However, parent Q_7 and Q_1 were good general combiner for grain yield. Parent Q_7 had significant negative GCA effects for plant and ear height, indicates dwarfness with low ear placement of the line. This parent possessed high mean values for grain yield and low values for plant and ear height also.

In the present study, parents were classified as high, average and low combiners based on their effects. Parents with desirable GCA effect (significantly different from zero) were considered as high combiners, while parents showing insignificant estimates were classified as average combiners. Low or poor combiners had significant but negative (undesirable) GCA effects.

Table 1. Analysis of variance for genotypic difference and combining ability for different characters in maize.

Source of variation	df	Mean of squares				
		Yield (t/ha)	Days to tasseling	Days to silking	Plant height (cm)	Ear height (cm)
Genotypes	28	2.10**	39.95**	33.75**	1115.18**	627.54**
GCA	6	1.16**	75.48**	57.20**	1461.62**	792.15**
SCA	21	2.49*	24.72**	23.70**	966.71**	556.99**
Error	56	0.07	0.91	1.10	42.556	39.89
σ^2A	-	0.24	9.02	5.95	87.98	41.81
σ^2D	-	1.44	14.11	13.40	547.65	306.43

*P<0.05, **P<0.01

Table 2. Estimates of GCA effects and mean values (in parenthesis) of parents for different characters in maize.

Parent/ inbred lines	Grain yield (t/ha)	Days to tasseling	Days to silking	Plant height (cm)	Ear height (cm)
Q_1	0.20*(4.12)	2.58**(83.3)	2.10**(85.3)	0.93(152.0)	-1.89(74.3)
Q_2	-0.21*(3.28)	-1.29**(84.0)	-0.61*(86.6)	-2.25(148.6)	-3.02(73.0)
Q_3	-0.26**(3.07)	-4.15**(82.6)	-3.76** (84.6)	0.69(151.0)	0.85(74.0)
Q_4	-0.06(3.38)	-0.62*(84.3)	-0.63*(87.6)	-7.18**(145.6)	-3.71*(70.0)
Q_5	0.15(3.87)	0.05(88.6)	-0.23(90.3)	-10.91**(141.3)	-3.89*(70.3)
Q_6	-0.27(3.14)	1.51**(90.3)	1.50**(93.3)	16.35**(157.6)	14.65**(76.6)
Q_7	0.46**(4.62)	1.53**(91.6)	1.24**(94.3)	-6.58**(144.0)	-8.42**(68.0)
SE *(gi)*	0.06	0.23	0.25	1.56	1.51
SE *(gi-gj)*	0.09	0.35	0.38	2.38	2.31
$LSD_{(5\%)}$	4.20	0.56	0.61	3.32	3.69

*P<0.05, **P<0.01

In case of days to tasseling and silking parents Q_2, Q_3, and Q_4 showed significant negative GCA effects and possessed low mean values for these traits. So they could be good combiner for earliness. For plant and ear height Q_4, Q_5, and Q_7 showed significant negative GCA effects and low mean values, indicates good combiner for short plant and low ear height. Negative estimates for plant and ear height are desirable. The comparison between mean performance and GCA effects of the parents showed a close relationship between them. Among the parents Q_7 was the highest yielder followed by Q_1 and these two parents also showed significant positive GCA estimates for grain yield (Table 2). Similar association between GCA and mean performance was reported by Hussain et al. (2003) and Ivy and Hawlader (2000). The parents Q_4, Q_5, and Q_7 with lower plant and ear height showed significant negative GCA effect for these traits. The good general combiners could effectively be used in future breeding program for development of high yielding hybrids with desirable traits.

Specific combining ability (SCA) effects

The estimates of SCA effects for yield and other traits are presented in Table 3. Out of 21 F_1s, seven crosses, viz. $Q_1 \times Q_2$, $Q_1 \times Q_6$, $Q_1 \times Q_7$, $Q_2 \times Q_3$, $Q_3 \times Q_4$, $Q_4 \times Q_6$ and $Q_6 \times Q_7$ showed significant positive SCA effects for yield. Positive SCA indicate that lines are in opposite heterotic groups, while negative SCA effects indicate lines are in the same heterotic group (Vasal et al., 1992). Among seven crosses, $Q_2 \times Q_3$ showed the highest positive SCA effect for yield had low × low combiners; $Q_1 \times Q_7$ had high × high; $Q_1 \times Q_6$ and $Q_6 \times Q_7$ involved of high × average; $Q_2 \times Q_5$ and $Q_3 \times Q_4$ had low × average and $Q_1 \times Q_2$ involved of high × low general combiners. Roy et al. (1998) noticed that the best crosses for yield and yield contributing characters involved of high × high, high × low, high × average and low × average general combiners for SCA effects in their study. Among seven crosses four involved of general combiners of which at least one parent was high. This result is supported by Vasal (1998), who suggested including one good combiner (especially female parent) during crossing to obtain higher heterosis. Aguiar et al. (2003) also pointed out similar opoinon that in the diallel analyses, one must select hybrids of highest specific combining ability in which one of the parental lines presents highest general combining ability. The observations of the above results indicated that additive × additive, additive × dominance and dominance × dominance gene interaction were responsible for derivation of good specific cross for higher grain yield.

For days to tasseling and silking, negative estimates are considered desirable as they are observed to be associated with earliness. Six crosses viz. $Q_1 \times Q_6$, $Q_1 \times Q_7$, $Q_2 \times Q_3$, $Q_2 \times Q_5$, $Q_3 \times Q_4$ and $Q_6 \times Q_7$ showed desirable significant negative SCA effects both for these two traits indicating to have earliness. On the other hand, five crosses viz. $Q_1 \times Q_2$, $Q_1 \times Q_3$, $Q_1 \times Q_5$, $Q_3 \times Q_4$ and $Q_4 \times Q_7$ showed desirable significant negative SCA effects both for plant and ear height, indicates dwarf type hybrids with low ear placement. In general, the GCA effects of the parents were not reflected in the SCA effects of the crosses in most of the studied traits. This result is supported by Debnath and Sarker (1987). Besides, Deitos et al. (2006) also suggested that good general combining parent does not always show high SCA effects in their hybrid combinations. The parents with good GCA for yield and negative GCA for plant and ear height and days to tasseling and silking may be extensively used in the hybridization program as a donor to obtain early and short statured hybrid with higher yield. The cross combinations showing high SCA effects for yield may be exploited.

Table 3. Estimates of SCA effects and percent standard heterosis for different characters in maize.

Cross combinations	Grain yield (t/ha)		Days to tasseling		Days to silking	
	SCA	H (%)	SCA	H (%)	SCA	H (%)
$Q_1 \times Q_2$	0.64**	3.64**	1.58**	-0.78	1.27*	0.77
$Q_1 \times Q_3$	-1.13**	-12.11**	3.78**	-1.55	3.13**	-1.16
$Q_1 \times Q_4$	-0.11	-1.33	0.18	-1.16	-0.03	-1.16
$Q_1 \times Q_5$	0.09	2.56	-0.10	-1.16	0.26	-0.39
$Q_1 \times Q_6$	0.30*	1.15	-2.56**	-2.33*	-2.47**	-1.54
$Q_1 \times Q_7$	0.33*	8.54**	-2.89**	-2.71**	-2.20**	-1.54
$Q_2 \times Q_3$	1.41**	9.71**	-4.36**	-15.50**	-4.20**	-12.36**
$Q_2 \times Q_4$	-1.88**	-17.60**	2.38**	-3.10**	3.33**	0.00
$Q_2 \times Q_5$	0.53**	1.89	-2.56**	-8.53**	-4.07**	-8.11**
$Q_2 \times Q_6$	-0.49**	-10.07**	1.31*	-2.33*	2.20**	1.16
$Q_2 \times Q_7$	-0.21	-0.36	1.64**	-1.94*	1.47**	0.00
$Q_3 \times Q_4$	0.72**	1.12	-4.42**	-14.34**	-2.80**	-11.20**
$Q_3 \times Q_5$	0.13	-0.85	1.31*	-7.36**	0.47	-6.95**
$Q_3 \times Q_6$	-0.77**	-12.20**	4.18**	-2.33*	3.40**	-1.54
$Q_3 \times Q_7$	-0.35	-1.84	-0.49	-7.75**	-0.01	-5.79**
$Q_4 \times Q_5$	0.19	0.90	0.71	-3.49**	0.01	-3.86**
$Q_4 \times Q_6$	1.17**	7.91**	-0.42	-3.10**	-1.40*	-3.47**
$Q_4 \times Q_7$	-0.09	0.56	1.58**	-0.78	0.87	-1.16
$Q_5 \times Q_6$	-0.80*	-8.79**	-1.02*	-3.49**	0.87	-0.39
$Q_5 \times Q_7$	-0.13	3.15	1.64**	-0.39	2.47**	1.16
$Q_6 \times Q_7$	0.59**	6.70**	-1.49**	-2.33*	-2.60**	-2.70**
$SE_{(ij)}$	0.12		0.45	0.93	0.49	0.85
$LSD_{(5\%)}$	7.27		0.97	1.93	1.05	1.77

Cross combi-nations	Plant height (cm)		Ear height (cm)	
	SCA	H (%)	SCA	H (%)
$Q_1 \times Q_2$	-10.51**	-13.34**	-13.33**	-24.28**
$Q_1 \times Q_3$	-11.44**	-12.42**	-12.20**	-19.94**
$Q_1 \times Q_4$	13.08**	-4.75*	14.60**	4.05
$Q_1 \times Q_5$	-12.18**	-18.10**	-8.80*	-21.10**
$Q_1 \times Q_6$	-2.44	-1.07	-5.33	-2.02
$Q_1 \times Q_7$	23.48**	0.31	25.07**	4.34
$Q_2 \times Q_3$	18.35**	-4.29*	16.60**	4.05
$Q_2 \times Q_4$	4.22	-14.42**	6.40*	-4.05
$Q_2 \times Q_5$	4.28	-16.10**	-5.67	-19.36**
$Q_2 \times Q_6$	-21.31**	-15.34**	-4.20	-2.02
$Q_2 \times Q_7$	4.96	-13.80**	0.20	-18.21**
$Q_3 \times Q_4$	-7.71*	-18.56**	-12.80**	-17.34**
$Q_3 \times Q_5$	-0.98	-17.18**	9.47**	-2.89
$Q_3 \times Q_6$	0.76	-3.83	2.93	7.51*
$Q_3 \times Q_7$	1.02	-14.26**	-4.00	-18.50**
$Q_4 \times Q_5$	11.89**	-14.88**	11.60**	-0.29
$Q_4 \times Q_6$	20.62**	1.69	2.07	7.51*
$Q_4 \times Q_7$	-42.11**	-37.73**	-21.87**	-33.24**
$Q_5 \times Q_6$	-6.64**	-12.58**	-1.33	-0.29
$Q_5 \times Q_7$	3.62	-18.40**	-5.27	-23.70**
$Q_6 \times Q_7$	9.02*	-3.37	5.86	2.02
$SE_{(ij)}$	3.07	1.94	2.98	2.74
$LSD_{(5\%)}$	6.59	4.04	6.39	5.71

*P<0.05, **P<0.01

Heterosis

Percent standard heterosis expressed by the F_1 hybrids over the check hybrid, Pacific 11 for yield and other traits are presented in Table 3. The degree of heterosis varied from cross to cross and from character to character. Heterosis ranged from -17.60 to 9.71%; -15.50 to -0.39%; -12.36 to 1.16%; -37.73 to 1.69% and -33.24 to 4.34% for grain yield, days to tasseling, days to silking, plant height and ear height, respectively.

Days to tasseling and days to silking determine the earliness or lateness of a hybrid. Negative heterosis is desirable for them. Significant negative heterosis both for days to tasseling and silking were observed in six crosses ($Q_2 \times Q_3$, $Q_2 \times Q_5$, $Q_3 \times Q_4$, $Q_3 \times Q_7$, $Q_4 \times Q_5$ and $Q_4 \times Q_6$). Earlier Maryam and Jones (1985) and Ganguli et al. (1989) reported negative heterosis for these traits in maize.

Negative heterosis is also desirable for plant height and ear height which helps for developing short statured plant leads to less lodging. Nine crosses showed significant negative heterosis for both of these traits. The highest significant negative heterosis for short plant height and low ear height was recorded by the cross $Q_4 \times Q_7$, while $Q_4 \times Q_6$ showed maximum positive heterosis for tallness and higher ear height. According to Singh (1979), generally earliness is associated with days to silking and the shorter plants with low ear height are associated with resistance to lodging.

Among the 21 F_1s, five crosses viz. $Q_1 \times Q_2$, $Q_1 \times Q_7$, $Q_2 \times Q_3$, $Q_4 \times Q_6$ and $Q_6 \times Q_7$ exhibited significant positive heterosis for grain yield. The highest heterosis 9.71% was shown by the cross $Q_2 \times Q_3$. Appreciable percentage of heterosis for grain yield in maize was also reported by Debnath (1992) and Roy et al. (1998).

From the study it can be concluded that, parents having good combining for yield (Q_1 and Q_7); earliness (Q_2, Q_3, and Q_4) and short plants (Q_4, Q_5 and Q_7) could be used as donor for obtaining high yield and desirable traits. In the present study both additive and non-additive gene actions were involved for grain yield and other traits. Therefore, for yield improvement in maize both additive and non-additive gene should be exploited through a suitable breeding program. The cross combinations $Q_1 \times Q_7$, $Q_2 \times Q_3$, $Q_4 \times Q_6$ and $Q_6 \times Q_7$ manifested significant high SCA effects coupled with excellent heterosis for yield could be used for hybrid development.

References

Aguiar, C. G., L. A. Carlini-Garcia, A. R. Silva, M. F. da Santos, A. A. F. Garcia and C. L. De Souja Jr. 2003. Combining ability of inbred lines of maize and stability of their respective single-crosses. Sci. Agric. 60:83-89.

Baker, R. J. 1978. Issues in a diallel analysis. Crop. Sci. 18:533-536.

Beck, D. L., S. K. Vaal and J. Carossa. 1990. Heterosis and combining ability of CIMMYT's tropical early and intermediate maturity maize (*Zea mays* L.) germplasm. Maydica. 35:279-285.

CropStat. 2007. Crop Research Informatics Laboratory. International Rice Research Institute. Los Banos, The Philippines.

Dass, S. V., P. Ahuja and M. Singh. 1997. Combining ability for yield in maize (*Zea mays* L.). Indian J. Genet. 57:98-100.

Debnath, S. C. 1992. Analysis of heterosis in a 10 × 10 diallel cross of maize. Bangl. J. Agric. Sci. 19:161-164.

Debnath, S. C. and K. R. Sarker. 1987. Genetic analysis of grain yield and some of its attributes in maize (*Zea mays* L.). Thai J. Agric. Sci. 20:263-276.

Deitos, A., E. Arnhold, F. Mora and G. V. Miranda. 2006. Yield and combining ability of maize cultivars under different eco-geografic conditions. Crop Breed. Appl. Biotech. Brazilian Soc. Plant Breed. 6:222-227.

Ganguli, D. K., M. F. Haque and P. K. Sinha. 1989. Heterosis in interpopulation crosses in maize. J. Res. 1:59-63.

Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci. 9:463-493.

Hussain, S. A., M. Amiruzzaman and Z. Hossain. 2003. Combining ability estimates in maize. Bangladesh. J. Agril. Res. 28:435-440.

Ivy, N. A. and M. S. Howlader. 2000. Combining ability in maize. Bangladesh J. Agril. Res. 25:385-392.

Khotyleva, L. V., L. S. Tarulina and I. Kapusta. 1986. Genetic interpretations of the combining ability of maize lines for quantitative character following use of different crossing systems. Biol. 8:78-82.

Maryam, B. and D. A. Jones. 1985. The genetics of maize (*Zea mays* L.) growing at low temperatures. II. Harvesting time, number of kernels and plant height at maturity. Euphytica 34:475-482.

Mathur, R. K. and S. K. Bhatnagar. 1995. Partial diallel cross analysis for grain yield and its component characters in maize (*Zea mays* L.). Ann. Agric. Res. 16:324-329.

Nass, L. L., M. Lima, R. Vencovsky, and P. B. Gallo. 2000. Combining ability of maize inbred lines evaluated in three environments in Brazil. Sci. Agric. 57:986-996.

Nigussie, M. and H. Zelleke. 2001. Heterosis and combining ability in a diallel among eight elite maize populations. Afr. Crop Sci. J. 9:471-479.

Patterson, H. D., E. R. Williams and E. A. Hunter. 1978. Block design for variety trials. J. Agric. Sci. (Cambridge). 90:395-400.

Rokadia, P. and S. K. Kaushik. 2005. Exploitation of combining ability for heterosis in maize (*Zea mays* L.). In: K. Pixley and S. H. Zhang. (Eds.). pp. 89-91. Proc. 9th Asian Reg. Maize Workshop. Beijing, China, September 5-9.

Roy, N. C., S. U. Ahmed, S. A. Hussain and M. M. Hoque. 1998. Heterosis and combining ability analysis in maize (*Zea mays* L.). Bangladesh J. Plant Breed. Genet. 11:35-41.

Singh, S. B. 1979. Genetic analysis for grain yield and other quantitative traits in inbred lines of maize (*Zea mays* L.). Ph.D. Dissertation, Banaras Hindu University. Baranasi. India.

Singh, R. K. and P. K. Singh. 1994. A manual on Genetics and Plant Breeding. Experimental Techniques. Kalyani Publs. Ludiana, New Delhi. pp.99-107.

Vasal, S. K. 1998. Hybrid maize technology: Challenges and expanding possibilities for research in the next century. In: S. K. Vasal, C. F. Gonzalez and F. Xingming. (Eds.). pp. 58-62. Proc. 7th Asian Reg. Maize Workshop. Los Banos, Philippines, February 23-27.

Vasal, S. K., G. Srinivasan., G. C. Han and C. F. Gonzalez. 1992. Heterotic patterns of eighty-eight white subtropical CIMMYT maize lines. Maydica. 37:319-327.

Zelleke, H. 2000. Combining ability for grain yield and other agronomic characters in inbred lines of maize (*Zea mays* L.). Indian J. Genet. 60:63-70.

Emir. J. Food Agric. 2013. 25 (2): 138-141
doi: 10.9755/ejfa.v25i2.10581
http://www.ejfa.info/

PLANT SCIENCE

Impact of silicon on various agro-morphological and physiological parameters in maize and revealing its role in enhancing water stress tolerance

Sajad Majeed Zargar[1*] and Abha Agnihotri[2]

[1]School of Biotechnology, SKUAST-J, Chatha, Jammu (Jammu & Kashmir) - 180009 (J &K) India
[2]Center for Agricultural Biotechnology, AIMT, Amity University, Uttar Pradesh, Block E-3, 4th Floor, Sector 125, Noida - 201 303 India

Abstract

Silicon (Si) is the second most abundant element in earth's crust and is considered as beneficial element for providing tolerance against various biotic and abiotic stresses. The aim of the present study was to investigate the role of silicon in water stress tolerance in maize as depicted through agro- morphological/ physiological parameters. We evaluated 15 diverse maize genotypes/ inbred lines under rain-fed conditions with, as well as without, Si application. Significant differences were observed in various agro-morphological and physiological parameters under Si^+ and Si^- conditions. The number of kernel per ear was significantly higher in Si+ conditions. The various physiological parameters observed include; anthesis- silking interval, relative water content, stomatal density and canopy temperature. All these parameters indicated the positive impact of Si on water stress tolerance in case of maize genotypes under study. These results specified that Si plays an important role in giving tolerance against drought/ water stress. The study can be further extended to investigate the molecular mechanism involved in Si induced water stress tolerance in maize.

Key words: Silicon, Maize, Drought, Abiotic stress tolerance

Introduction

Maize (*Zea mays* L.) is an important cereal and fodder crop occupying 27% of the world acreage and accounts for about 34% of the world grain production. It is world's third most important cereal crop while drought is second important constraint of maize production in the developing countries. Drought affects the organ growth and leads to reduction in leaf and silk elongation hence decrease in light interception and increase in anthesis-silking interval, finally affecting corn production (Boyer, 1970; Saab and Sharp, 1989). Many of the world's poorest people farm in areas with inadequate rainfall. The major global research focus to overcome the moisture stress is a real challenge for the scientists as it leads to extensive yield losses.

The role of various macro- and micro- nutrients in enhancing the yield has been understood very well. The role of silicon (Si) on drought tolerance was examined by Hattori et al. (2005) in *Sorghum bicolor*, their results clearly indicated that the Si applied sorghum could extract a large amount of water from drier soils and can maintain a higher stomatal conductance. Similar results were obtained by Ma and co-workers in 2004 in cucumber; they concluded that Si enhances the net photosynthetic rate of cucumber under drought stress. Si has also been found to reduce the oxidative membrane damage and improves the water use efficiency up to 35% in maize (Gao et al., 2004). Kaya et al. (2006) reported that in case of maize, on application of Si, relative water content was enhanced, indicating retention of water in cells that increases moisture stress tolerance. They also proposed that Si plays important role in regulation of calcium concentration in plants which maintains membrane integrity in water stressed plants. Liang (1999) observed that Si maintains optimum supply of essential nutrients that may be due to change in soil pH. Pei et al. (2010) reported that in case of wheat, grown in water stress conditions, Si application stimulates antioxidant defense. Kvedaras et al. (2007) have shown that the Si does not only enhance the tolerance to water stress but also improves the resistance of sugarcane plants

Received 17 January 2012; Revised 26 March 2012; Accepted 06 May 2012; Published Online 28 November 2012

*Corresponding Author

Sajad M. Zargar
School of Biotechnology, SKUAST-J, Chatha, Jammu (Jammu & Kashmir) - 180009 (J &K) India

Email: smzargar@gmail.com

to insect attack (Stalk borer). Gong et al. (2006) determined that the application of silicates in the saline culture improves the growth of shoots and correlated it with the Si deposition in the exodermis and endodermis in rice (*Oryza sativa* L.) seedlings. Hence the present study was undertaken to understand and divulge the role of Si in inducing water stress tolerance in maize.

Material and Methods

Plant material

Fifteen diverse genotypes (inbreed lines) of maize (Table 1) were procured from Indian Agriculture Research Institute (IARI), New Delhi and evaluated at The Energy and Resources Institute (TERI) New Delhi, experimental field station at Gaul Pahari, Gurgaon (Haryana) India.

Field Evaluation

The sowing of these genotypes was done in the first week of July in a randomized block design. The genotypes were evaluated under rain-fed conditions with and without application of Si. Each of the fifteen genotypes was replicated twice under both Si^+ and Si^- conditions. Calcium silicate (Ca_2SiO_4) fertilizer was applied in soil before sowing as a source of silicon at the rate of 700Kg per hectare. All other standard cultivation practices were followed except irrigation and the experiment was conducted under rain-fed conditions. The experiment was conducted to reveal potential of Si in enhancing tolerance against moisture stress, which was provided by growing plants in rainfed condition.

Agro-morphological and physiological observations

Various agro-morphological parameters (germination percentage, plant height, days to maturity, and kernels per ear) and physiological parameters (anthesis-silking interval, canopy temperature, stomatal density and relative water content) were recorded to evaluate the differences among these parameters due to application of calcium silicate/ silicon. The observations were recorded from five randomly selected plants of each genotype in both replications in Si fortified as well as controlled (Si^-) conditions.

Recording of various physiological observations

Anthesis-silking interval

The date of anthesis as well as silking was recorded and the interval was calculated:

Anthesis Silking interval = Date of anthesis – Date of silking Or Date of silking- Date of anthesis.

Canopy temperature

Canopy temperature was recorded from 5 plants of each genotype in each replication using Infra-Red Thermometer (9V Infra-Red Thermometer Gun).

Stomatal density

Epidermal impression was prepared by coating the leaf surface with nail polish which was peel off, once nail polish was dried, it was mounted onto a slide by a cello tape. The impression approach was used to determine leaf stomatal density using AIM Research Binocular Microscope, (Make - AIM Scientific) which was expressed as the number of stomata per unit leaf area (Radoglou and Jarvis, 1990).

Relative water content

It determines the amount of water that is retained in cells and is calculated by:

RWC (%) = [(W-DW) / (TW-DW)] x 100

Where, W – sample fresh weight; TW – sample turgid weight; DW – sample dry weight

The weight was done using electronics balance (Microbalance – Sartorius - BT - 224S).

Statistical analysis

Experiment was laid in 2 X 4 factorial Randomized Block Design (RBD). Analysis of variance was performed by online statistical package (Web Agri Stat Package-WASP1) of ICAR-Goa Regional Centre, Goa, India. Least significant difference (LSD) was calculated and means were compared. Same package calculated CV, by using formula CV=(Standard deviation/mean)X 100.

Table 1. List of maize genotypes evaluated for water stress tolerance.

Sr. No.	Genotype	Sr. No.	Genotype
1	MGU 201	9	MGU 209
2	MGU 202	10	MGU 210
3	MGU 203	11	MGU 211
4	MGU 204	12	MGU 212
5	MGU 205	13	MGU 213
6	MGU 206	14	MGU 214
7	MGU 207	15	MGU 215
8	MGU 208		

Table 2. Impact of silicon on agro-morphological traits under water stress conditions.

Trait/Treatment	Germination %	Plant height	Days to maturity	Kernels per ear
Silicon treated (Si^+)	80.967	1.967	1.870	1.954**
Control (Si^-)	78.400	1.983	1.868	1.656
CD (5%)	4.534	0.189	0.191	0.294
CV	10.777	18.116	19.322	30.816

CD (Critical difference at 5% level of significance); CV (Coefficient of variation); ** (indicates significant difference in the trait due to application of silicon); All observations, except germination %, were transformed to logarithmic values and analyzed

Results and Discussion

We observed significant difference for number of kernels per ear under Si fortified and controlled conditions at 30 % coefficient of variation (Table 2). These findings are in line with the earlier reports by Elawad and Green (1979) and Savant et al. (1997), who have observed that soluble Si enhances the growth, development and yield of several plant species including equisetum, rice, sugarcane, wheat, and some dicotyledonous species.

The germination percentage and plant vigor was higher under Si fortified conditions as compared to control but it was non-significant. Further, no significant difference was observed for plant height and days to maturity under Si fortified and controlled conditions (Table 2). Gong et al. (2003) have reported increase in the plant height in case of wheat grown in pots with Si under well watered conditions, however no significant changes in the dry materials under drought conditions were observed on application of Si.

To examine the impact of silicon on water stress tolerance, various physiological observations were recorded; anthesis silking interval, relative water content, stomatal density and canopy temperature. Under Si applied conditions the anthesis-silking interval and canopy temperature was lesser and significantly different from the controlled conditions (Table 3). As indicated in the earlier studies (Boyer, 1970; Saab and Sharp, 1989) drought leads to reduction in leaf and silk elongation hence decrease in light interception and increase in anthesis-silking interval finally affecting corn production. Hence lesser the anthesis-silking interval, more water stress tolerant will be the genotype; and thus lesser anthesis-silking interval due to Si application suggests the role of Si in providing water stress tolerance in maize. The relative water content under Si applied conditions was higher and significantly different from control. Gong et al. (2003) have observed that in case of wheat grown under water stress conditions under Si applied conditions maintain higher relative water content and water potential.

In the present study, we have not observed any significant difference for the stomatal density among plants grown under Si^+ and controlled conditions (Table 3). Gao et al. (2006) have also reported that Si does not affect the stomatal morphology and density, and it is presumed that Si plays a role in decreasing the transpiration rate by changing the stomatal movement rather than its density. Similar to this hypothesis, significant difference was observed for canopy temperature among maize genotypes for Si^+ and Si^- conditions under the present study.

Our results indicate that Si induces tolerance against moisture stress. The relative water content and canopy temperature are the two standard parameters to differentiate drought tolerant genotypes. To the best of our knowledge this is the first report to provide an evidence of decrease in the canopy temperatures in maize due to Si application. These preliminary findings supported by the past results indicate that Si not only enhances tolerance to water stress but also increases yield in case of maize.

Table 3. Impact of silicon on physiological parameters under water stress conditions.

Trait/Treatment	Anthesis silking interval	Canopy temperature	Stomatal density	Relative water content
Silicon treated (Si^+)	0.606**	1.338**	1.672	66.167**
Control (Si^-)	0.700	1.354	1.665	61.167
CD (5%)	0.090	0.011	0.018	1.383
CV	26.129	11.610	2.022	4.114

CD (Critical difference at 5% level of significance); CV (Coefficient of variation); ** (indicates significant difference in the trait due to application of silicon); All observations, except germination %, were transformed to logarithmic values and analyzed

Conclusion

Si induced water stress tolerance has been studied in several crops. In our study we have seen the impact of Si on various agro-morphological and physiological parameters in maize. Our preliminary studies have shown that application of Si does not only improve the agronomic traits but also improves the physiological parameters corresponding to drought tolerance in maize. Further work is needed to understand the genetic mechanism behind Si induced water stress tolerance in maize.

Acknowledgement

The work was carried out at TERI, New Delhi (India) under the DRDO sponsored project of Dr. Agnihotri. We sincerely thank Dr. Prasanna, IARI, New Delhi (India) for providing Maize genotypes for this study and DRDO, New Delhi, India for financial support.

References

Boyer, J. S. 1970. Leaf enlargement and metabolic rates in corn, bean and sunflower at various leaf water potential. Plant Physiol. 46:233-235.

Elawad, S. H. and V. E. Green. 1979. Silicon and the rice plant environment: A review of recent research. Riv. Riso. 28:235-253.

Gao, X., C. Zou, L. Wang and F. Zhang. 2006. Silicon decreases transpiration rate and conductance from stomata of maize plants. J. Plant Nutr. 29(9):1637-1647.

Gong, H., K. Chen, G. Chen, S. Wang and C. Zhang. 2003. Effects of silicon on growth of wheat under drought. J. Plant Nutr. 26(5):1055-1063.

Gong, H. J., D. P. Randall and T. J. Flowers. 2006. Silicon deposition in the root reduces sodium uptake in rice (*Oryza sativa* L.) seedlings by reducing bypass flow. Plant Cell Env. 29:1970-1979.

Gao, X., C. Zou, L. Wang and F. Zhang. 2004. Silicon improves water use efficiency in maize plants. J. Plant Nutr. 8(27):1457-1470.

Hattori, T., S. Inanaga, H. Araki, P An, S. Morita, M. Luxová and A . Lux. 2005. Application of silicon enhanced drought tolerance in *Sorghum bicolor*. Physiol. Plant. 123(4):459-466.

Kaya, C., L. Tuna and D. Higgs. 2006. Effect of silicon on plant growth and mineral nutrition of maize grown under water stress conditions. J. Plant Nutr. 29:1469-1480.

Kvedaras, O. L., M. G. Keeping, F. R. Goebel and M. J. Byrne. 2007. Water stress augments silicon mediated resistance of susceptible sugarcane cultivars to the stalk borer *Eldana saccharina* (Lepidoptera: Pyralidae). Bull. Ent. Res. 97:175-183.

Liang, Y. 1999. Effect of silicon on enzyme activity and sodium, potassium and calcium concentration in barley under salt stress. Plant Soil. 209:217-224.

Ma, C., Q. Li, Y. Gao and T. Xin. 2004. Effect of silicon application on drought resistance of cucumber plants. Soil Sci.nd Plant Nutr. 50:623-632**.**

Pei, Z. F., D. F. Ming, D. Liu, G. L. Wan, X. X. Geng, H. J. Gong and W. J. Zhou. 2010. Silicon improves the tolerance of water deficit stress induced by polyethylene glycol in wheat (*Triticum aestivum* L.) seedlings. J. Plant Growth Regul. 29:106-115.

Radoglou, K. M. and P. G. Jarvis. 1990. Effects of CO_2 enrichment on four poplar clones. II. Leaf surface properties. Ann. Bot. 65:627-632.

Saab, I. N. and R. E. Sharp. 1989. Non-hydraulic signals from maize roots in drying soil: inhibition of leaf elongation but not stomatal conductance. Planta 179:466-474.

Savant, N. K., G. H. Snyder and L. E. Datnoff. 1997. Silicon Management and Sustainable Rice Production. In: D. L. Sparks (Ed.) pp.151-199, Advances in Agronomy, vol. 58. Academic Press, San Diego, CA.

Emir. J. Food Agric. 2013. 25 (2): 142-149
doi: 10.9755/ejfa.v25i2.10649
http://www.ejfa.info/

ANIMAL SCIENCE

Enrichment of protein content in cassava (*Manihot esculenta* Crantz) by supplementing with yeast for use as animal feed

Sineenart Polyorach[1], Metha Wanapat[1]* and Sadudee Wanapat[2]

[1]Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand
[2]Department of Plant Science and Natural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

Abstract

The aim of this investigation was to study the kinetics of yeast *S. cerevisiae* growth in different media and to study the enrichment of cassava chip by fermentation using yeast. Experiment was conducted according to a 2x4 factorial arrangement in a Completely Randomized Design (CRD) to study growth kinetics of Baker's yeast (YB) and Brewer's yeast (YC) cultivated in 4 media with following composition: (urea:molass:water) = 40:32:100 (M1), 48:24:100 (M2), 56:16:100 (M3), 64:08:100 (M4) at pH 3.5-5.0 at 27°C for 120 h. The kinetic data of yeast growth were collected at 0 and every 6 h post-cultivation. All treatments were selected from the optimum cultivation time (66 h) mixed with cassava chip at ratio of 1 ml:1.3 g. For yeast fermented cassava chip protein (YEFECAP) products were analyzed for proximate composition. The results showed that kinetic growth of *S. cerevisiae* in YBM3 was the highest in number (3.0×10^{11} cell/ml) at 66 h post-cultivation. However, YEFECAP production in YBM4 (Baker's yeast with urea: molasses: water at ratio 64:08:100 (M4)) was highest in protein content at 47.5% crude protein CP when compared to other treatments. Furthermore, further research is required to study the use of YEFECAP as a protein source to replace soybean meal in *in vivo* feeding trials especially in productive ruminants.

Key words: *Saccharomyces cerevisiae*, Baker's yeast, Brewer's yeast, Yeast fermented cassava chip protein (YEFECAP), Protein enrichment, Cassava chip

Introduction

Cassava or tapioca (*Manihot esculenta*, Crantz) is an annual tropical tuber crop grown widely in tropical and sub-tropical countries (Wanapat, 2003). This plant is easily grown under minimal management and it adapts to poor soil conditions, low rainfall, high temperature and pest tolerance (Salami and Akintokun, 2008). Usually, cassava is grown for root production as energy sources. Cassava tuber can be processed into dried chip which consists of soluble carbohydrate 76-81%, but is low in crude protein (1-3% CP) depending on cultivar. Moreover, *S. cerevisiae* has been widely used for protein production (Lang et al., 1997).

Eukaryotic microorganisms can be considered a suitable host for the production because firstly, growth of microorganism is very much fast, secondly, a broader range of materials may be considered as suitable substrates depending on the microorganism chosen. The two chief strategies with regard to substrate to consider are: low grade waste material, or to use relatively simple carbohydrate source to produce microbial material containing very high quality of protein (Reed and Nagodawithana, 1995). Urea is a low cost fertilizer (Al-Moshileh et al., 2005), varying concentration of urea were added as nitrogen supplement for yeast growth. Ali et al. (2009) showed that urea could support maximum microbial biomass protein production. Furthermore, molasses contains readily utilizable carbohydrates available in the form of fermentable sugars and can be used for yeast growth (Mukhtar et al., 2010; Polyorach et al., 2011), almost 75% of the world's molasses come from sugarcane grown in tropical climates of Asia and South America (Piggot, 2005). The process of protein enrichment of animal feed using the microorganism to improve the nutritional value of cassava has been evaluated (Oboh, 2006). This method of upgrading the protein content of cassava has been developed. Recently, Oboh and

Received 23 January 2012; Revised 13 May 2012; Accepted 20 May 2012; Published Online 28 November 2012

*Corresponding Author

Metha Wanapat
Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

Email: metha@kku.ac.th

Akindahinsi (2003) (reported that *S. cerevisae* could also be used for enriching cassava products. Boonnop et al. (2009) demonstrated that supplementation of cassava chip with Baker's yeast (*S. cerevisae*) could increase crude protein from 2% to 32.4%. Moreover, Brewer's yeast (*S. cerevisae*) is by-product that can be produced in association with the production of beer, one of interesting microorganism used for enrichment of animal feed. Therefore, the objectives of this study were as follows: (a) To determine the optimal media for faster growth of yeast and (b) To determine which yeast-media combination would enhance crude protein content in cassava.

Materials and Methods

Treatments and experimental design

Eight treatments combination were randomly assigned according to a 2x4 factorial arrangement in a Completely randomized design (CRD) to study growth kinetics of two yeast type (*S. cerevisiaes*); 1. Baker's yeast (YB), 2. Brewer's yeast (YC) cultivated in four media with differing ratios of urea:molass:water (g/g/ml) = 40:32:100 (M1), 48:24:100 (M2), 56:16:100 (M3), 64:08:100 (M4).

Materials

The commercial Baker's yeast (*S. cerevisiae*) and Brewer's yeast (*S. cerevisiae*), manufactured by Berly Speciality Industries and by-product from Khon Kaen Brewery Co., Ltd. respectively were used in the fermentation processes. Cassava chip, commercial grade urea and sugar cane molasses were purchased from the local shop.

Yeast cultivation

Preparation of activated yeast: 20 g of Baker's yeast/ Brewer's yeast was weighed and put into a flask and added 20 g cane sugar and 100 ml distilled water, mixed well and incubated at room temperature for 1 h (A). Liquid media preparation: 24 g molasses was weighed and dissolved in 100 ml distilled water, followed by addition of 48 g urea and then adjusted pH of the solution using H_2SO_4 to achieve a final pH 3.5-5 (B). Mixed (A) and (B) at 1:1 ratio then flushed with air for 120 h at room temperature by using air pump (600 W). Yeast fermented liquid was sampled at 0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78, 84, 90, 96, 102, 108, 114, 120 h post-cultivation.

Yeast fermented cassava chip protein (YEFECAP) production

Choosed the best time (66 h) of cultivation, transfer yeast media solution mixed with cassava chip ratio at 1 ml : 1.3 g, then dried under shade for 72 h, and followed by sun-drying for 48 h. Final products were stored in plastic bag for mixing in the concentrate.

Determination of yeast growth

The growth kinetics of yeasts were determined by using regression equation (Figure 1.) from standard curve between the absorbance (Ao) at 600 nm and number of cells (cell/ml) from concentration of Baker's yeast/Brewer's yeast dry mass at 0.0003, 0.0006, 0.0009, 0.0012, 0.0015 and 0.0018 g/ml. Yeast fermented samples were measured the absorbance of the samples with the spectrophotometer at 600 nm (Bausch and Lomb spectrophotometer, VWR Scientific Inc. N.Y.) and then used regression equation for determined yeast number (cell/ml) from absorbance.

Chemical analysis

Dry matter (DM) of yeast fermented cassava chip protein (YEFECAP) at different level of liquid media were analyzed by drying at 100 °C for 12 h in a hot air oven, ash, ether extract (EE) and crude protein (CP) determined according to AOAC (1995). The samples were also analyzed for neutral-detergent fiber (NDF) and acid-detergent fiber (ADF) according to Van Soest et al. (1994). YBM4 was analyzed for amino acid profile at Central Laboratory (Thailand) Co., Ltd (In house method based on AOAC Official Method 994.12, 988.15v (2000).

Statistical analysis

Kinatics of yeast growth and chemical composition of YEFECAP were statistically analyzed using analysis of variance of a Completely randomized design with a 2x4 factorial arrangement using Proc. GLM procedure (SAS, 1998). Treatment means were statistically compared using Duncan's New Multiple Range Test (Steel and Torrie, 1980).

Results

Standard curves

The standard curves were produced from 8 treatment combinations at 0 h. Figure 1. shows the relationship between absorbance values and number of cells. It was found that when increasing concentration of yeast, absorbance value and the number of cells were increased. The linear relationship between cell numbers and absorbance values are illustrated in Figure 1. The values of r^2 of YBM1,YBM2,YBM3,YBM4,YCM1,YCM2,YCM 3 and YCM4 were 0.92, 0.97, 0.96, 0.98, 0.92, 0.98, 0.98 and 0.94, respectively. This result implied that regression equation could use to determine yeast number (cell/ml) from absorbances.

Table 1. Kinetic of growth of the different type of yeasts and different mediums in this experiment (x 10^{11} cell/ml).

Cultivation	YB				YC					Contrast[1]		
Time (h)	M1	M2	M3	M4	M1	M2	M3	M4	SEM	A	B	A*B
0	0.5	0.4	0.5	0.5	0.4	0.4	0.5	0.4	0.07	ns	ns	ns
6	0.5	0.5	0.6	0.6	0.5	0.4	0.5	0.4	0.07	ns	ns	ns
12	0.6	0.6	0.8	0.6	0.6	0.5	0.6	0.6	0.09	ns	ns	ns
18	0.8^{ab}	0.8^{ab}	0.9^{ab}	0.9^{a}	0.7^{ab}	0.6^{b}	0.6^{ab}	0.6^{b}	0.09	**	ns	ns
24	1.0^{a}	1.1^{a}	1.1^{a}	1.0^{ab}	0.7^{b}	0.7^{b}	0.7^{b}	0.7^{b}	0.10	**	ns	ns
30	1.1^{ab}	1.2^{a}	1.2^{a}	1.1^{a}	0.9^{ab}	0.8^{b}	1.1^{ab}	1.1^{ab}	0.08	*	ns	ns
36	1.4	1.6	1.4	1.3	1.3	1.2	1.2	1.1	0.13	ns	ns	ns
42	1.4^{c}	1.9^{a}	1.8^{ab}	1.5^{bc}	1.6^{bc}	1.6^{abc}	1.7^{abc}	1.4^{c}	0.10	ns	*	ns
48	1.9	2.2	2.3	1.9	1.8	2.0	2.0	1.9	0.12	ns	ns	ns
54	2.2^{bc}	2.6^{ab}	2.7^{a}	2.2^{bc}	2.0^{c}	2.4^{abc}	2.4^{abc}	2.0^{c}	0.13	ns	*	ns
60	2.2^{de}	2.8^{ab}	3.0^{a}	2.3^{cde}	2.1^{e}	2.5^{bcd}	2.7^{abc}	2.2^{de}	0.12	ns	**	ns
66	2.1^{c}	2.7^{ab}	3.1^{a}	2.2^{c}	2.1^{c}	2.5^{bc}	2.8^{ab}	2.2^{c}	0.14	ns	**	ns
72	2.1^{bc}	2.7^{abc}	3.0^{a}	2.2^{bc}	2.1^{c}	2.5^{abc}	2.8^{ab}	2.2^{bc}	0.19	ns	**	ns
78	2.2^{c}	2.7^{ab}	3.1^{a}	2.2^{c}	2.1^{c}	2.5^{bc}	2.8^{ab}	2.2^{c}	0.13	ns	**	ns
84	2.1^{c}	2.7^{ab}	3.0^{a}	2.2^{c}	2.1^{c}	2.5^{bc}	2.8^{ab}	2.2^{c}	0.14	ns	**	ns
90	2.1^{d}	2.7^{b}	3.0^{a}	2.2^{cd}	2.1^{d}	2.5^{bc}	2.8^{b}	2.2^{d}	0.08	*	**	ns
96	2.1^{c}	2.7^{ab}	3.0^{a}	2.2^{c}	2.1^{c}	2.5^{bc}	2.7^{ab}	2.2^{c}	0.13	ns	**	ns
102	2.1^{d}	2.7^{bc}	3.0^{a}	2.2^{d}	2.1^{d}	2.5^{c}	2.7^{b}	2.2^{d}	0.07	*	**	ns
108	2.1^{e}	2.7^{bc}	3.0^{a}	2.2^{de}	2.0^{e}	2.5^{cd}	2.7^{b}	2.2^{e}	0.08	*	**	ns
114	2.1^{d}	2.7^{b}	3.0^{a}	2.2^{d}	2.0^{d}	2.4^{c}	2.7^{b}	2.1^{d}	0.06	**	**	ns
120	2.1^{cd}	2.7^{ab}	3.0^{a}	2.2^{cd}	2.0^{d}	2.4^{bc}	2.7^{abc}	2.2^{cd}	0.11	ns	**	ns

abcde Means with different superscripts differ (P<0.05), [1] A=effect of yeast, B=effect of medium, A*B= interaction between yeast and medium, YB= Baker's yeast, YC= Brewer's yeast, M1= 40: 32: 100 (urea: molasses: water), M2= 48: 24: 100, M3= 56: 16: 100, M4= 64: 08: 100, SEM= Standard error of the mean, ns= Non-significant difference, *P<0.05, **P<0.01. (Calculated from regression equation).

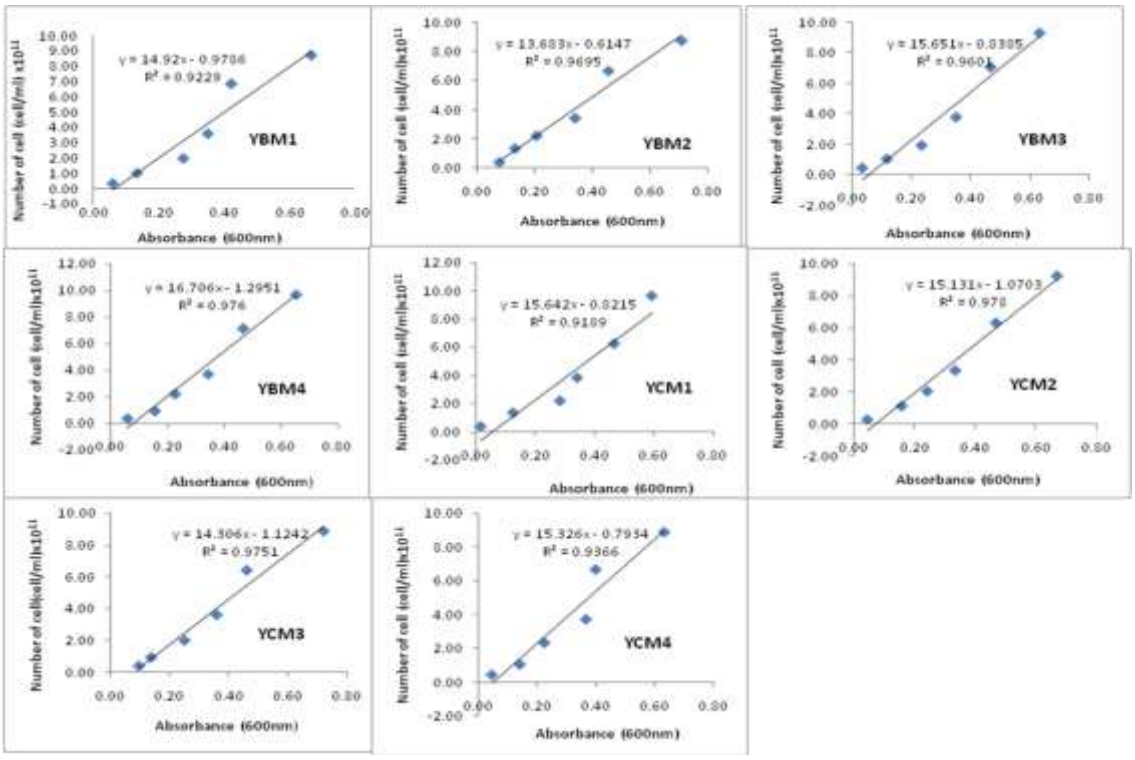


Figure 1. Standard curve and regression equation of each treatment combinations.

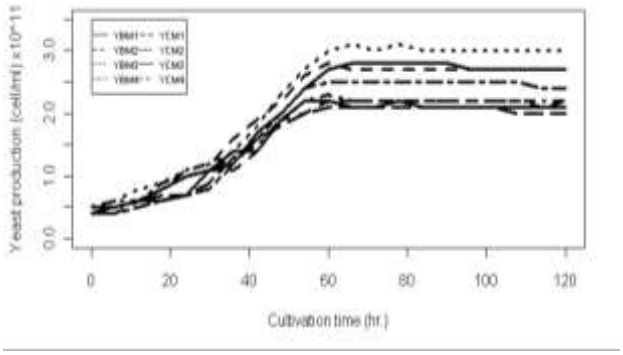


Figure 2. Effects of different yeast type and mediums on kinetics of yeast growth.

Effects of different yeast type and different medium on kinetic of yeast growth

Baker's yeast and Brewer's yeast were cultured in plastic bucket containing 5000 ml of yeast fermented liquid at pH between 3.5 to 5.0 at 27°C (Table 1 and Figure 2). It was shown that most of the treatments resulted in highest growth starting from 60 h post-cultivation except for YBM3 and YCM3 started from 66 h post-cultivation. At 66 h post-cultivation. YBM3 was the highest ($P<0.05$) in number at $3x10^{11}$cell/ml while YBM1 and YCM1 were the lowest ($P<0.05$) in number $2.1x10^{11}$cell/ml.

Effect of medium on kinetic of yeast growth

Figure 3 shows the growth curve of yeast *S. cerevisiae* (Baker's yeast and Brewer's yeast) in medium M1, M2, M3 and M4 (urea: molasses: water; M1=40:32:100, M2=48:24:100, M3=56: 16:100 and M4=64:08:100). It showed significant differences ($P<0.05$) at 42, 54, 60, 66, 72, 78, 84, 90, 96, 102, 108, 114 and 120 h post-cultivation by yeast growth of medium M1, M2 and M3 were increased ($P<0.05$) when increasing proportion of urea, and the highest ($P<0.05$) in medium M3, while in medium M4 resulted in a poor result.

Effect of yeast type on kinetic of yeast growth

Effect of yeast type on kinetic of yeast growth is shown in Figure 4. It was found that growth of Baker's yeast (YB) was significantly ($P<0.05$) higher than Brewer's yeast (YC), especially at 18, 24, 30, 90, 102, 108 and 114 h post-cultivation.

Effects of different yeast type and medium on chemical composition of YEFECAP products

Effects of different yeast type and medium on chemical composition of yeast fermented cassava chip protein (YEFECAP) product are shown in Table 2. It was found that interaction between yeast type and medium was significantly ($p<0.01$) influenced on percentage of DM, OM, CP and ADF. DM of YBM1 had the highest DM (92.1%) and YCM1 and YCM2 were the lowest (90.1%), while OM of YBM3 was the highest (97.3%) and YCM4 was the lowest (96.0%). The mean protein value of YEFECAP products; YBM4 was the highest followed by YBM3, YCM4, YBM2, YCM3, YCM2, YBM1 and YCM1 and the values were 47.5, 46.4, 41.9, 40.6, 38.5, 37.4, 33.7 and 29.3 respectively. However, there were no changes ($P>0.05$) in EE and NDF contents of the YEFECAP products. Amino acid profile of YBM4 is shown in Figure 5. It was shown that lysine was the highest followed by leucine, glutamic acid, isoleucine, valine, tyrosine, alanine, aspartic acid, histidine, glycine, proline, threonine, methionine and tryptophan respectively, while arginine, cystine and hydroxylysine were the lowest.

Discussion

Standard curves

Percentage of variation in response of cell/ml to factors of absorbance value measured by spectrophotometer and the cell numbers of direct cell count were closely related accordingly. Therefore, the regression equations between spectrophotometer and direct counts from standard curves could be used in this experiment.

Table 2. Chemical composition of YEFECAP (% of DM).

	YB				YC					Contrast[1]		
Item	M1	M2	M3	M4	M1	M2	M3	M4	SEM	A	B	A*B
DM	92.1^{a}	90.9^{b}	90.6^{bc}	90.6^{bc}	90.1^{c}	90.1^{c}	90.3^{c}	91.9^{b}	0.16	**	**	**
OM	96.7^{cd}	96.9^{c}	97.3^{a}	97.2^{ab}	97.2^{ab}	97.1^{b}	96.6^{d}	96.0^{e}	0.05	**	**	**
CP	33.7^{g}	40.6^{d}	46.4^{b}	47.5^{a}	29.3^{h}	37.4^{f}	38.5^{e}	41.9^{c}	0.28	**	**	**
EE	7.3	8.7	9.9	7.9	8.4	9.6	9.7	8.7	0.69	ns	ns	ns
NDF	6.9	6.8	6.7	6.1	6.6	6.5	6.0	6.4	0.16	ns	ns	ns
ADF	4.5^{c}	4.1^{e}	3.5^{f}	4.3^{d}	5.3^{a}	4.9^{b}	4.0^{e}	4.5^{c}	0.06	**	**	**

abcdefgh Means with different superscripts differ ($P<0.05$), [1] A=effect of yeast, B=effect of medium, A*B= interaction between yeast and medium, YB= Baker's yeast, YC= Brewer's yeast, M1= 40: 32: 100 (urea: molasses: water), M2= 48: 24: 100, M3= 56: 16: 100, M4= 64: 08: 100, SEM= Standard error of the mean, ns= Non-significant difference, **$P<0.01$.

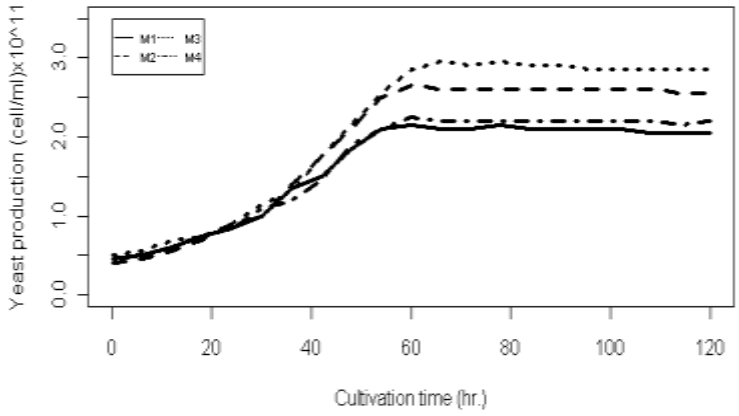


Figure 3. Effects of mediums on kinetics of yeast growth.

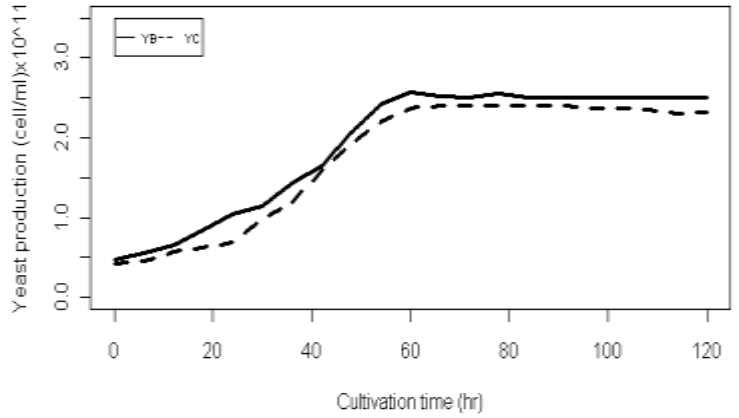


Figure 4. Effects of yeast type on kinetics of yeast growth.

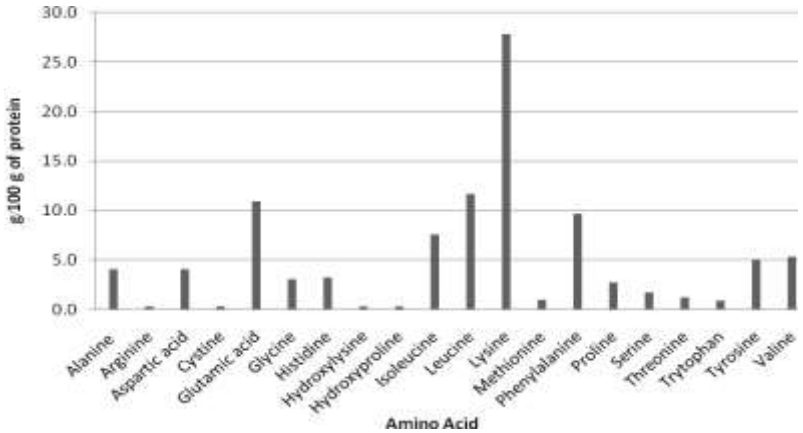


Figure 5. Amino acid profile of YEFECAP products in YBM4 (Baker's yeast with urea: molasses: water ratio at 64:08:100 (M4)).

Effects of different yeast type and medium on kinetic of yeast growth

Concerning kinetic of growth of Baker's yeast and Brewer's yeast, it was shown that in the first 0-6 h from the beginning, the growth was slow because the cells were adapted to the new surrounding medium. The volume of cells was swelling and the metabolism of cell was increased, but the proliferation of cell was slow, as a lag phase. After lag phase, the growth became rapidly increased because cells can adapt to the surrounding medium, and the substances can pass

through cell than in the lag phase. Hence, the growth of yeast was increased by budding cells in this phase, as an exponential phase. The budding cell cycle is the succession of events, whereby a cell grows and divides into two daughter cells that each contains the information and then, repeats the process. The pH and temperature in this study were similar with the previous work, the optimum pH levels for *S. cerevisae* cultivation were from 3.5 to 6.0 and temperature levels were from 20 to 40 °C (Wang et al., 2004; Pramanik and Roa, 2005; Asli, 2010; Manikandan and Viruthagri, 2010).

In this study most of the treatments resulted in the highest growth starting from 60 h post-cultivation except for YBM3 and YCM3 started from 66 h post-cultivation. At 66 h post-cultivation YBM3 was highest in number at $3x10^{11}$cell/ml while YBM1 and YCM1 were lowest in number $2.1x10^{11}$cell/ml, that could be an effect of the from different medium ($p < 0.05$). At the same time of cultivation yeast and interaction between yeast type and medium had no affect ($P>0.05$) on yeast numbers. This result agreed with the work of Wang et al. (2004), Pramanik and Roa (2005) and Asli (2010) who reported that optimal cultivation time of *S. cerevisiae* was approximately 60 to 80 h by highest biomass.

Effect of mediums on kinetic of yeast growth

The growth curve of yeast *S. cerevisiae* (Baker's yeast and Brewer's yeast) in medium M1, M2 and M3 with increasing proportion of urea, yeast growth were higher and the highest in medium M3, while medium M4 resulted in poor result. Manikandan and Viruthagiri (2010) reported that optimum carbon and nitrogen ratio (C:N) of cultivation medium of *S. cerevisiae* was found to be 35.2 which yielded a maximum ethanol and cell mass. Whereas, Danesi et al. (2006) used yeast extract as nitrogen source and molasses as carbon source, it was found that optimal C:N ratio for recombinant *S. cerevisiae* cultivation was 10.

In this study, the poor growth in medium M4 could be explained that the concentration of urea was not optimum, and large amount of urea might exert a toxic effect. An appropriate amount of C:N ratio was the key to harvest maximum microbial biomass protein (Zheng et al., 2005). However, generally the results confirmed that urea, a low cost fertilizercould supported maximum microbial biomass protein production which confirmed from previous findings (Ali et al., 2009). Furthermore, utilization of sucroses or glucose as carbon source is not economical in the production of microbial biomass protein and a less expensive carbohydrate source would be beneficial. Low cost substrates such as cane molasses can be used for the production of microbial biomass protein for animal feed supplements (Sattar et al., 2008). Molasses, a cheap by-product, is widely available from the sugar industry and consist of water, sucrose (47-50%, w/w) which is the disaccharide most easily utilized by yeast cells, 0.5-1% of nitrogen source, proteins, vitamins, amino acids, organic acids and heavy metals (Roukas, 1998). Hence, it is a very attractive carbon source for microbial biomass protein production by mixed culture from economic point of view.

Effect of yeast type on kinetics of yeast growth

The growth of Baker's yeast (YB) was higher ($P<0.05$) than Brewer's yeast (YC). That could be due to Brewer's yeast is by product from beverage industry which might contain some of waste product from yeast metabolism which could inhibit yeast growth. However, potential of *S. cerevisiae* (Baker's yeast and Brewer's yeast) has been widely used for protein production (Lang et al., 1997). For the two yeast, they showed fast growth, short generation time, a border range of materials may be considered as suitable substrates depending on the microorganism chosen especially, can be grown on media containing cheap sources of C and N for efficient yield (Reed and Nagodawithana, 1995).

Effects of different yeast type and medium on chemical composition of YEFECAP products

The increase in growth and proliferation of fungi or bacterial complex in the form of single cell proteins may possibly account for the apparent increase in the protein content and also found by Oboh (2006). Moreover, Aro (2008) reported that microbial fermentation with cassava starch residue could improve nutritive quality by increasing CP, EE and ash and decreased cyanide, CF, NDF and ADF. This high protein content could be attributed to the ability of the *S. cerevisiae* to secret some extracellular enzymes such as amylases, linamarase and cellulase in to cassava mash during their metabolic activities, which would lead to yeast growth (Oboh and Akindahunsi, 2003). The protein contents of product (Table 2) were higher than that reported by Boonnop et al. (2009) and Wanapat et al. (2011), it could be due to particle size of cassava chip was smaller and also yeast fermented liquid and cassava ratio was higher than those reported by Boonnop et al. (2009) and Wanapat et al. (2011). YBM4 contained essential amino acid especially lysine, this result is similar with Yamada and Sgarbieri (2005) reported that whole cell of *S. cerevisiae* was high in amino acid profile especially

lysine. Even though, kinetic of yeast growth in YBM3 in this study was the highest ($P<0.05$) but when considering the crude protein content of YEFECAP products, it was lower than in YBM4. It could be due to NPN level in YBM4 that was higher than in YBM3, which is a good N source use for synchronized soluble carbohydrates in the rumen of ruminants (NRC, 2001; Wanapat, 2003; Wanapat and Khampa, 2007). Moreover, many researchers studied about synchronizing urea and cassava chip by processing a product such as cassadro (Poungchompu, 2000) and U-cal (Cherdthong, 2010, 2011).

Conclusions and Recommendations

Based on the results of this experiment, it could be concluded that growth kinetic of *Sacchromyces cerevisiae* in YBM3 (Baker's yeast with urea:molasses:water ratio at 56:16:100 (M3)) was the highest in number ($3.0x10^{11}$ cell/ml) at 66 h of cultivation. However, YEFECAP products in YBM4 (Baker's yeast with urea: molasses: water ratio at 64:08:100 (M4)) was the highest in protein content at 47.5% CP with high in lysine. However, further research is required to study the use of YEFECAP as a protein source to replace soybean meal in *in vivo* feeding trials in productive ruminants.

Acknowledgements

The authors would like to express their most sincere thanks to all who have assisted and supported the research in this study, particularly the Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand, the Royal Golden Jubilee Ph. D. Scholarship Program, Khon Kean Brewery Co., Ltd. for providing financial support of research and the use of research facilities.

References

Al-Moshileh, A. M., M. A. Errebhi and M. I. Motawei. 2005. Effect of various potassium and nitrogen rates and splitting methods on potato under sandy soil and arid environmental conditions. Emir. J. Agric. Sci. 17(1):01-09.

Ali, S., S. Ahmed, M. A. Sheikh, A. S. Hashmi, M. I. Rajoka and A. Jamil. 2009. Lysine production by L-homoserine resistance mutant of *Brevibacterium flavum*. J. Chem. Soc. Pak. 31:97-102.

AOAC. 1995. Official Methods of Analysis, Animal Feeds. 16th Edn, Association of Official Analysis Chemists, Virginia, U.S.A. pp: 1-8.

Aro, S. O. 2008. Improve in the nutritive quality of cassava and its by-products through microbial fermentation. Afr. J. Biotechnol. 7:4789-4797.

Asli, M. S. 2010. A study on some efficient parameters in bach fermentation of ethanol using *Saccharomyces cerevesiae* SC1 extracted from fermented siahe sardasht pomace. Afr. J. Biotechnol. 9:2906-2912.

Boonnop, K., M. Wanapat, N. Nontaso and S. Wanapat. 2009. Enriching nutritive value of cassava root by yeast fermentation. Sci. Agric. (Piracicaba, Braz.) 66:616-620.

Cherdthong, A., M. Wanapat and C. Wachirapakorn. 2010. Influence of urea calcium mixture supplementation on ruminal fermentation characteristics of beef cattle fed on concentrates containing high levels of cassava chips and rice straw. Anim. Feed Sci. Technol. 163:43–51.

Cherdthong, A., M. Wanapat and C. Wachirapakorn. 2011. Effects of urea–calcium mixture in concentrate containing high cassava chip on feed intake, rumen fermentation and performance of lactating dairy cows fed on rice straw. Livest. Sci. 136:76-84.

Denesi, E. D. G., A. S. M. Miguel, C. D. O. Reangel-Yagui, J. C. M. Carvalho and D. A. P. Jr. 2006. Effect of cerbon:nitrogen ratio (C:N) and substrate source on glucose-6-phosphate dehydrogenase (G6PDH) production by recombinant *Saccharomyces cerevisiae*. J. Food Eng. 75:96-103.

Lang, C., C. Gollnitz, M. Popovic and U. Stahl. 1997. Optimization of fungal polygalacturonase synthesis by *Saccharomyces cerevisiae* in fed-batch culture. Chem. Eng. J. 65:219-226.

Manikandan, K. and T. Viruthagiri. 2010. Optimization of C/N ratio of the medium and Fermentation conditions of Ethanol Production from Tapioca Starch using Co-Culture of *Aspergillus niger* and *Saccharomyces cerevisaie*. Int. J. Chem. Tech. Res. 2:947-955.

Mukhtar, K., M. Asgher, S. Afghan, K. Hussain and S. Zia-ul-Hussnain. 2010. Comparative

Study on Two Commercial Strains of *Saccharomyces cerevisiae* for Optimum Ethanol Production on Industrial Scale. J. Biomed. Biotechnol. doi:10.1155/2010/419586. National Research Council. 2001. Nutrient Requirements of Dairy Cattle, 7th revised ed. National Academic Science, Washington, DC, USA.

NRC, 2001. Nutrient Requirements of Dairy cattle. 7th Revised Edition, National Academy Press, Washington, D. C.

Oboh, G. and A. A. Akindahinsi. 2003. Biochemical changes in cassava products (flour & gari) subjected to *Sacchromyces cerevisiae* solid media fermentation. Food Chem. 82:599-602.

Oboh, G. 2006. Nutrient enrichment of cassava peels using a mixed culture of *Sacchromyces cerevisiae* and *Lactobacillus* spp. solid media fermentation technique. Electr. J. Biotechnol. 9:46-49.

Piggot, R. 2005. Treatment and fermentation of molasses when making rum-type spirits. The Alcohol Text book. Nottingham University Press, London, UK.

Poungchompu, O., M. Wanapat, C. Wachirapakorn and N. Nontaso. 2000. Effect of Ruminal Infusion of Starch-Urea (Cassadro) Solution in Swamp Buffaloes Fed on Urea-Treated Rice Straw. In: Proceedings of the 9th Congress of the AAAP and 23rd Biennial Conference of the ASAP, vol. B, July 3-7, 2000. University of New South Wales, Sydny, Australia. p. 234.

Polyorach, S., M. Wanapat, C. Wachirapakorn, C. Navanukraw, S. Wanapat and N. Nontaso. 2011. Supplementation of Yeast Fermented Liquid (YFL) and coconut oil on rumen fermentation characteristics, N-balance and urinary purine derivatives in Beef cattle. J. Anim. Vet. Adv. 10(16):2084-2089.

Pramanik, K. and D. E. Roa. 2005. Kinatic study on ethanol fermentation of grape waste using *Sacchromyces cerevisiae* yeast isolated from toddy. Insitute Engineering India J. 85: 53-58.

Reed, G. and T. Nagodawithana. 1995. Biotechnology enzymes, biomass, food and feed. Bibiliographic Citation. 9:168-215.

Roukas, T. 1998. Pretreatment of beet molasses to increase pullulan production. Proc. Biochem. 33:805-810.

Salami, A. O. and A. K. Akintokun. 2008. Post-harvest enzymatic activities of healthy and infected Cassava (*Manihot esculenta* Crantz) tubers. Emir. J. Food Agric. 20(1):01-17.

SAS. 1998. SAS/STAT User's Guide. Version 6.12. SAS Inst. Inc., Cary, NC.

Sattar, M., S. Ahmed, M. A. Sheikh and A. S. Hashmi. 2008. Fermentation of yeast sludge with *Brevibacterium flavum* to enhance lysine concentration. J. Chem. Soc. Pakistan. 30:642-648.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometerial Approach, second ed. McGraw-Hill, New York, U.S.A.

Van Soest, P. J. 1994. Nutritional ecology of the ruminant, second ed. Coenell University, NY. p 476.

Wanapat, M. 2003. Manipulation of cassava cultivation and utilization to improve protein to energy biomass for livestock feeding in tropics. Asian. Australas. J. Anim. Sci. 16:46-52.

Wanapat, M. and S. Khampa 2007. Effect of levels of supplementation of concentrate containing high levels of cassava chip on rumen ecology, microbial N supply and digestibility of nutrients in beef cattle. Asian. Australas. J. Anim. Sci. 20:75–81.

Wanapat, M., S. Polyorach, V. Chanthakhoun and N. Sornsongnern. 2011. Yeast-fermented cassava chip protein (YEFECAP) concentrate for lactating dairy cows fed on urea–lime treated rice straw. Livest. Sci. 139:258-263.

Wang, K., S. Vavassori, L. M. Schweizer and M. Schweizer. 2004. Impaired PRPP-synthesizing capacity compromises cell integrity signalling in *Saccharomyces cerevisiae*. Microbiol. 150:3327–3339.

Yamada, E. A. and V. C. Sgarbieri. 2005. Yeast (Saccharomyces cerevisiae) protein concentrate: preparation, chemical composition, and nutritional and functional properties. J. Agric. Food Chem. 53:3931-3936.

Zheng, Y. G., X. L. Chen and Z. Wang. 2005. Microbial biomass production from rice straw hydrolyses in airlift bioreactor. J. Biotechnol. 118:413-420.

Emir. J. Food Agric. 2013. 25 (2): 150-158
doi: 10.9755/ejfa.v25i2.15402
http://www.ejfa.info/

ANIMAL SCIENCE

Effect of ageing on meat quality of the one humped camel (*Camelus dromedarius*)

O. M. A. Abdelhadi[1*], S. A. Babiker[2], J. F. Hocquette[3], B. Picard[3], D. Durand[3] and Bernard Faye[4]

[1]*Department of Animal Production, Faculty of Natural Resources & Environmental Studies, University of Kordofan, Sudan*
[2]*Department of Meat Production, Faculty of Animal Production, University of Khartoum, Sudan*
[3]*INRA, UR1213, Herbivore Research Unit, 63122 Theix, France*
[4]*CIRAD, UR 18, Campus International de Baillarguet, 34398 Montpellier cedex 5, France*

Abstract

A total of seven she-camels (3-4 years old) were slaughtered following the normal abattoir procedures in Khartoum state, Sudan. Longissimus thoracis (LT) muscle samples were aged for 1, 3, 5, and 7 days at 1-3° C. Chemical composition, muscle pH, drip loss (DL), water holding capacity (WHC), color, myosin heavy chain isoforms (MyHC) as well as lipid peroxidation (MDA) and vitamin E content were determined at the indicated times during ageing. Ageing time of LT muscle influenced significantly ($P<0.001$) chemical composition except ash. Dry matter and drip losses significantly increased while moisture and protein contents decreased during ageing, however, no differences were found in muscle pH, color, fat peroxidation and WHC although fat peroxidation tended to increase from 5 days of ageing onwards. Electrophoresis of MyHC isoforms indicated the presence of two muscle fiber types only: type I (64.1%) and type IIa (35.9 %), respectively. In conclusion, shelf life of camel meat could be extended in the presence of high levels of vitamin E which helps to maintain lipid stability.

Key words: Ageing, Desert she-camel, Longissimus thoracis, Lipid peroxidation

Introduction

Ageing of meat is a highly complex phenomenon which depends on physicochemical parameters, extent and rate of acidification, changes in osmotic pressure, glycolytic and proteolytic enzymes (Ouali, 1991). After slaughtering, there is a decrease in temperature and pH (Marsh et al., 1988) and an increase in osmotic pressure (Bonnet et al., 1992) and expressible juiciness (Offer and Knight, 1989). Consequently, there is a weakening of the myofibrillar structure and an improvement in tenderness of the cooked beef (Ho et al., 1996; Wegner et al., 2000). Several studies showed that ageing improves the tenderness of most muscles (Campo et al., 2000). The post-mortem ageing of meat is a very important period having a significant effect on its microstructure and quality traits, especially texture, tenderness and water-holding capacity (Zamora et al., 1996).

Camel meat is the least studied type of meat and is wrongly believed to be of lower nutritive value and quality than other types of red meat, inspite of their ability to produce good quality meat at comparatively low cost under extremely harsh environments. In addition, there is lack of research in the improvement of camel meat characteristics (Skidmore, 2005). Investigation of muscle fiber characteristics is of practical importance to meat scientists, breeders, and the meat industry to provide a better understanding of the involvement of muscle fibers with regard to the determination of muscle growth and final meat quality traits (Wegner et al., 2000). Lipid peroxidation is one of the major causes of quality deterioration in raw and cooked meat products during refrigerated or frozen storage. Peroxidation occurs when lack of balance between production of reactive oxygen species (ROS) and the level of antioxidants take places (Favier, 1997). It takes place in polyunsaturated fatty acids (PUFA), the most susceptible being FA of the n-3 family (n-3 PUFA) initiated by (ROS), which are essential for life and growth in animal cells (Kama–Eldin and Yanishlieva, 2002). Lipid peroxidation in meat, with production of malondialdehyde (MDA) and other oxidized products are toxic for consumers

Received 07 March 2012; Revised 05 April 2012; Accepted 31 May 2012; Published Online 28 November 2012

*Corresponding Author

O. M. A. Abdelhadi
Department of Animal Production, Faculty of Natural Resources & Environmental Studies,
University of Kordofan, Sudan

Email: abusin911@yahoo.com

and harmful to human health (Sasaki et al., 2001; Alderton et al., 2003). The presence of antioxidants prevents peroxidation from happening (Burton and Inglold, 1989). Among antioxidants, vitamin E (as α tocopherol acetate) was reported controlling efficiently lipid oxidation and thus extends shelf life of meat (Arnold et al., 1993; Faustman and Wang, 2000; Kerry et al., 2000). Thus, lipid stability is one of the important factors for maintaining meat quality during storage. The main objective of the present study is to investigate the effect of post-mortem ageing on chemical composition and quality characteristics of *longissimus thoracis* (LT) muscle of one humped camels (*Camelus dromedarius*).

Materials and Methods

Collection of samples

A total of seven she-camels (3-4 years old) fattened by local camel herders in Khartoum state, Sudan were slaughtered following the normal abattoir procedures. Samples of *Longissimus thoracis* (LT) muscle between the 5^{th} to 10^{th} rib were obtained from the right side of the carcasses after 60 minutes post slaughter, placed in plastic bags and transported to meat science laboratory, Faculty of Animal production, University of Khartoum in an insulated box filled with ice. In the laboratory, any visible fat was trimmed and each muscle was then divided into 4 parts, aged for 1, 3, 5, and 7 days at 1-3°C. Samples were then vacuumed, packed and stored at –18°C awaiting the analysis.

Meat quality evaluation

Quality characteristics of *longissimus thoracis* muscles included: pH, water holding capacity (WHC), drip loss (DL) and meat color coordinates (*l*, a*, b**). Muscle pH was measured 60 min post slaughter, 1, 3, 5 and 7 days postmortem using a portable pH meter (Hanna waterproof pH meter, Model H I 9025, Italy) with temperature adjusting probe inserted at same depth each time into the muscle. Water holding capacity (WHC) was expressed as percentage of the expelled water using filter paper method as the total wetted area less than meat film area (cm^2) relatively to the sample weight (g). Drip loss was calculated from the difference in muscle weight before and after ageing. Meat color co-ordinates *l** (lightness), *a** (red-green) and *b** (yellow-blue) were measured using a Minolta CR100 chromameter (Minolta Co., Ltd., Japan).

Chemical analysis

The chemical composition of the LT muscle (dry matter, protein, intramuscular fat and ash) was determined according to the standard methods of AOAC (1990). Crude protein was determined using a Foss Tecator Kjeltec 2300 Nitrogen/Protein Analyzer. Intramuscular fat was determined by Soxhlet extraction of the wet sample, using petroleum ether. Ash content was determined by ashing samples in a muffle furnace at 500°C for 24 h. The results were expressed on dry matter bases.

Electrophoresis of myofibrillar proteins

Muscle fibre types were determined by quantifying the different myosin heavy chain (MyHC) isoforms using Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Talmadge and Roy (1993). Bovine albumin was used as a reference sample. Each hole of gel was loaded with 4-5 µg of myofibrillar protein and electrophoresis was performed at a constant voltage of 70 volts for 30 hours at 4°C. The relative proportions of slow isoforms (% MyHC I) and fast isoforms (% MyHC IIa) were determined after staining of gels in a solution of Coomassie Blue R250 and quantifying using Image Quant TL v2003.

Malondialdehyde (MDA) determination

As a marker of lipid oxidation intensity, MDA was extracted from LT muscle homogenized powder (1g) by the procedure of Botsoglou et al. (1994). Briefly, 5 ml of 0.8% butyl hydroxytoluene in hexane and 8 ml of 5% thiobarbituric acid in H_2O were added to camel meat sample. After crushing and adding 2 ml of hexane, the mixture was centrifuged for 10 min at 10,900 g to recover the aqueous phase. Then 0.7 ml of 5% trichloroacetic acid and 1.5 ml of 0.8% thiobarbituric acid were added to the previously filtered aqueous phase. The sample was heated in a water bath at 70°C for 30 min, cooled in an ice bath and, after addition of 1 ml n-butanol, centrifuged for 10 min at 680g to recover the complex MDA-TBA_2 present in the upper phase.

Meat MDA content was determined by HPLC as described by Agarwal and Chase (2002). An aliquot (180 µl) of this solution was injected into an HPLC system (Perkin–Elmer Instruments, Shelton, Connecticut) equipped with an RP C18 column (ODB 5 lm, 4.6 250 mm, Interchim, Montluçon, France). The mobile phase (flow rate: 0.6 ml/min) was composed of potassium phosphate (0.05 M, pH 6.8) in methanol. MDA was detected by fluorescence (excitation at 515 calibration curve).

Determination of α tocopherol acetate content

α -tocopherol acetate was assayed by High Performance Liquid Chromatography (HPLC) and Ultraviolet (UV) absorption as described by Scislowski et al. (2005). Before the extraction step, α -tocotrienol acetate (22.5 µg/ml) was added as internal standard to muscle powder samples to determine extraction efficiency. Powder of camel LT muscle (1 g) was treated by heating at 80 C° in 11% KOH in ethanol for 20 min and α- tocopherol was extracted twice with 5 ml of hexane. After elimination of hexane by evaporation, α-tocopherol and α-tocotrienol acetate were dissolved in 160 µl of tetrahydrofurane and 240 µl of methanol/dichloromethane (65/35, vol/ vol). Finally, α-tocopherol and α-tocotrienol concentrations were determined by UV spectrophotometer (Kontron, model 430) at 292 nm using Kroma System 2000 software (Kontron Instruments, Zurich, Switzerland).

Statistical analysis

Data were subjected to statistical analysis to test the effect of ageing time (days) on camel meat characteristics by ANOVA using general linear model procedure of SAS (2001). Least squares means were calculated and significance was declared at *(P<0.05*). Principal Component Analysis (PCA) was used to interpret the relationship between chemical analysis (dry matter, moisture, protein, fat and ash), muscle pH, color co-ordinates (*a*, l** and *b**), WHC, drip loss, vitamin E and lipid peroxidation (total: 15 variables). They were carried out taking into account the most explicit variables for each analysis to work out inter-relationships between all the parameters. The p variables observed on the 7 (n) camels were represented by projection in the main plan constituted by the two first factor axes. These factor axes were calculated from the eigenvectors of the p variables and the correlation matrix of the data (Jobson, 1992).

Results and Discussion

Chemical composition

The overall mean of moisture, dry matter, crude protein, intramuscular fat and ash content were: 74.9, 25.1, 19.9, 4.8 and 1.2 respectively in *longissimus thoracis* (LT) muscle (Table 1), which within the range reported by Dawood and Alkanhal (1995), Al-Owaimer (2000), Al-Ani (2004), Kadim and Mahgoub (2006) and Kadim et al. (2008) for moisture (70-77)%, intramuscular fat (0.5-9.8)%, ash content (1-1.3)% but slightly lower than the range of crude protein (20-23)%. Significant differences were observed in chemical composition during ageing time in moisture, dry matter, crude protein and intramuscular fat, however no differences were found in ash content. Moisture content was found to decrease significantly during ageing which is associated with significant increase in dry matter. Crude protein was significantly lower in day 7 compared to days 1, 3 and 5 which could be attributed to escape of proteins in the purge or drip during ageing. This was confirmed previously by Savage et al. (1990) who indicated significant amount of protein lost from postmortem muscle; most of them are water-soluble, sarcoplasmic proteins. Intramuscular fat showed some inconsistent variations between time points which may be explained by sampling variations.

Table 1. Chemical composition of *longissimus thoracis* muscle (LT) from desert she-camels during ageing.

Parameters	Ageing time				SEM	Effect of cold storage *(P- value)*
	1	3	5	7		
Moisture (%)	76.0^{a}	75.1^{b}	74.6^{bc}	73.7^{c}	0.22	0.0004
Dry matter (%)	23.9^{c}	25.0^{b}	25.4^{ab}	26.3^{a}	0.22	0.0004
Crude protein (%)	20.6^{a}	20.2^{ab}	19.5^{ab}	19.1^{b}	0.21	0.0463
Intramuscular fat (%)	4.23^{ac}	6.8^{a}	2.86^{c}	5.61^{ab}	0.50	0.02
Ash (%)	1.12	1.15	1.16	1.18	0.02	0.37

a,b,c means with different superscripts within a row are significantly different at (P<0.05).

Table 2. Quality characteristics of aged *longissimus thoracis* (LT) muscle from desert female camels.

Parameters	Ageing time (Days)				SEM	Effect of cold storage *(P- value)*
	1	3	5	7		
Muscle pH	5.72	5.59	5.58	5.58	0.024	0.08
Muscle color:						
l*	27.50	27.59	28.13	29.26	0.59	0.73
a*	12.98	14.01	13.77	13.18	0.29	0.57
b*	14.08	15.34	14.76	15.55	0.30	0.33
Drip loss	1.58^{c}	3.37^{b}	4.24^{ab}	5.51^{a}	0.40	0.0001
WHC	1.36	1.28	1.22	0.95	0.10	0.51
MyHC isoforms (%):						
Ia	67.32	63.40	63.57	62.08	1.70	0.75
IIa	32.68	36.60	36.92	37.92	170	0.75
MDA (µg/ g muscle)	0.08	0.08	0.14	0.24	0.03	0.14
Vitamin E (µg/ g muscle)	16.39	24.45	12.80	18.35	2.01	0.39

a,b,c means with different superscripts within a row are significantly different at (P<0.05).

Meat quality of camel meat

The overall mean of muscle pH during ageing was 5.63 (Table 2) which were higher than the values reported by Soltanizaheh et al. (2008) who indicated that pH of 5.5, and lower than Kadim, Mahgoub et al. (2009) who reported pH of 5.79 in camel LT muscle. These differences could be attributed to the glycogen storage in the muscle at the time of slaughter. The pH declined rapidly to 5.72 in day one and then in slow rate until the ultimate point. This goes in accordance with the fact that camels are animals with a high gluconeogenesis capacity due to the presence of hump. The amount of enzymes in its glycolytic pathway causes slower glycogen degradation and pH decline (Immonen and Puolanne, 2000; Soltanizaheh et al., 2008). No significant difference was found in pH values during ageing of muscles which fits with the results of Kadim et al. (2009) who found no significant effect of ageing on ultimate pH (Table 2).

Muscle color development could be influenced by many factors (Table 2) including: myoglobin concentration, ultimate pH, muscle fiber type and cooling rate (Faustman and Cassens, 1990). The overall mean values of color coordinates *(l*, a* and b*)* during ageing were 28.1, 13.5 and 14.9, respectively indicating that meat from camel was moderately brighter and less red. These findings were in agreement with Kadim et al. (2009) who reported no significant effect in muscle color during ageing time.

Drip loss is a result of shrinkage of the myofibrils due to postmortem drop of pH and attachment between thin and thick filaments at the onset of rigor mortis and denaturation of myosin. Ageing affected drip loss significantly ($P<0.001$), however, no differences were observed between day 3 and 5 on one hand, day 5 and 7 on the other hand (Table 2). This goes in line with the fact that drip loss could increase linearly with a decrease in the length of the sarcomeres in muscle cells (Honikel et al., 1986).

Water holding capacity (WHC) is an important meat quality characteristic due to its influence on the nutritional value, appearance, palatability and processing properties of meat (Offer and Knight, 1988a; Lawrie, 1979). Many factors were reported previously affecting WHC (Hamm, 1986; Offer and Knight, 1988a,b; Honikel, 2004; Puolanne and Halonenm, 2010). In the present results, the overall mean of WHC was 1.2; however it decreased from 1.36 in day one to 0.95 in day 7 with no significant difference among ageing days. This indicated that an improvement in WHC which agreed with Lawrie (1979) who stated that WHC improved with ageing.

Myosin heavy chain isoforms

Ageing is the process that causes an increase in tenderness over time and involves specific degradation of structural proteins (Hwang et al., 2003). Electrophoresis of myosin heavy chain isoforms (SDS-PAGE) indicated the presence of two different muscle fiber types (slow MyHC I and fast MyHC IIa) in camel (LT) muscle, (Table 2 and Figure 1) as previously described (Abdelhadi et al., 2012). The fast isoforms (MyHC IIx and MyHC IIb), described in bovine muscle (Picard and Cassar-Malek, 2009) have not been revealed in all the studied samples.

The mean percentages of type I and type IIa were 64.1 and 35.9 %, respectively. This generally goes in line with the results of Kadim et al. (2009)

and Rose et al. (1992) who observed a higher proportion of type I compared to type IIa in (LT) muscle of breeding and racing camels. Tenderness is related to the high percentage of type I (with a high oxidative activity) which is able to use mainly fatty acids as energy-yielding nutrients. This agreed with Calkins et al. (1981), Dransfield et al. (2003) and Jurie et al. (2007) who reported that the oxidative capacity of a muscle was related to marbling and/or tenderness. No significant effect of ageing on the proportion of MyHC fiber types in she-camel (LT) muscle.

Lipid peroxidation and Vitamin E

Several factors can affect lipid oxidation in meat during ageing and packing including light, oxygen concentration, temperature, presence of anti- and pro-oxidants and abundance of unsaturated fatty acids (Jakobsen and Bertelsen, 2000). The overall mean of Malondialdehde (MDA) level in LT muscle was 0.14 µg/ g muscle. Low MDA values revealed during ageing of LT muscle in day 1 and 3 (0.08 µg/ g), respectively, however, these values increased to 0.14 and 0.24 µg/ g in day 5 and 7. No differences in MDA levels among ageing days which goes in line with the findings of Durand et al. (2006) from Charolais cull cows aged for 5 days and Durand et al. (2010) who reported MDA level was not modified by ageing time from 1 to 12 days whatever the ageing method (entire carcass *vs.* under vacuum).

Vitamin E (α-tocopherol) is an important lipid-soluble antioxidant that protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This would remove the free radical intermediates and prevent the oxidation reaction from continuing. The antioxidant function is considered to be the most important function of this vitamin (Bell, 1987; Herrera and Barbas, 2001; Traber and Atkinson, 2007). No significant effect of ageing on vitamin E during ageing days was found. The overall mean of vitamin E in the present study was three fold higher (17.8 µg/ g) than that reported in bovine rumsteack (5 µg/ g) by Durand et al. (2007 and 2010) in LT muscle of cows fed with plant extracts combined with vitamin E. Thus, camel meat could be a good source of vitamin E to fulfill the recommended daily requirements (15 mg/ day) by the Food and Nutrition Board (2000). MDA values were low in day 1 and 3 (0.08 µg/ g) and high in day 5 and 7 (0.14 and 0.24 µg/ g) which could be related to vitamin E levels in day 1 and 3 (16.4 and 24.5 µg/ g) as well as in the later (12.8 and 18.4 µg/ g) and to the low vitamin E values in day 5 and 7. These findings goes in line with Fausman and Wang (2000) and Kerry et al. (2000) since vitamin E is considered to control lipid oxidation in meat from many animal species (Table 2). To our knowledge no previous reports were found describing the effect of ageing of camel LT muscle on lipid peroxidation and vitamin E content in camel meat.

Principal component analysis (PCA)

During ageing there was a decrease in moisture, protein, ash and an increase in drip loss with no effect on fiber type I and IIb, lipid peroxidation and vitamin E. The Principal component analysis (PCA) explained about 36.1% of the total variance which can be condensed in two new principal components: PC1 and PC2 which explained 21.4 and 14.7% of the variance, respectively (Figure 2a). As shown in Fig. 2b, PC1 discriminates the ageing groups of muscles according to time (days). PC2 discriminates muscle samples aged for 1 and 7 days from those aged for 3 and 5 days. However, PC2 discriminates samples mainly on their fiber type proportions. High correlation coefficient was found between intramuscular fat and ash ($r= 0.90$, $P<0.05$), moisture and fat ($r=0.67$, $P<0.05$) and moisture with ash ($r=0.58$, $P<0.05$). However, negative correlation coefficient was found between drip loss and muscle protein; intramuscular fat and ash contents, respectively (-0.48, -0.73 and -0.74). Lipid peroxidation was negatively correlated with muscle fibers type I ($r= -0.40$) which could be attributed to the oxidative activity of type I muscle fibers (use fatty acids to obtain energy) (Jurie et al., 2002; Oury et al., 2007). Also dry matter showed negative correlation with intramuscular fat and ash (-0.67 and -0.58), as well as muscle pH and muscle fibers type IIa ($r= -0.42$). The proportions of types I and IIa muscle fibers as well as moisture and dry matter content were negatively correlated ($r= -1.0$, $P<0.05$). It could be concluded that ageing had decreased moisture, improved WHC and increased drip loss, dry matter, lipid peroxidation with no effect of ageing on fiber type and vitamin E.

Conclusion

Chemical composition and drip loss in LT muscle of camels were significantly affected by ageing. Inspite of the insignificant differences, lipid peroxidation tended to increase in days 5 to 7 which could indicate high risk of ageing meat from this type that contained high levels of intramuscular fat longer than 7 days. High levels of vitamin E in camel meat compared to bovine would help to increase shelf life of camel meat products.

Figure 1a **Figure 1b**

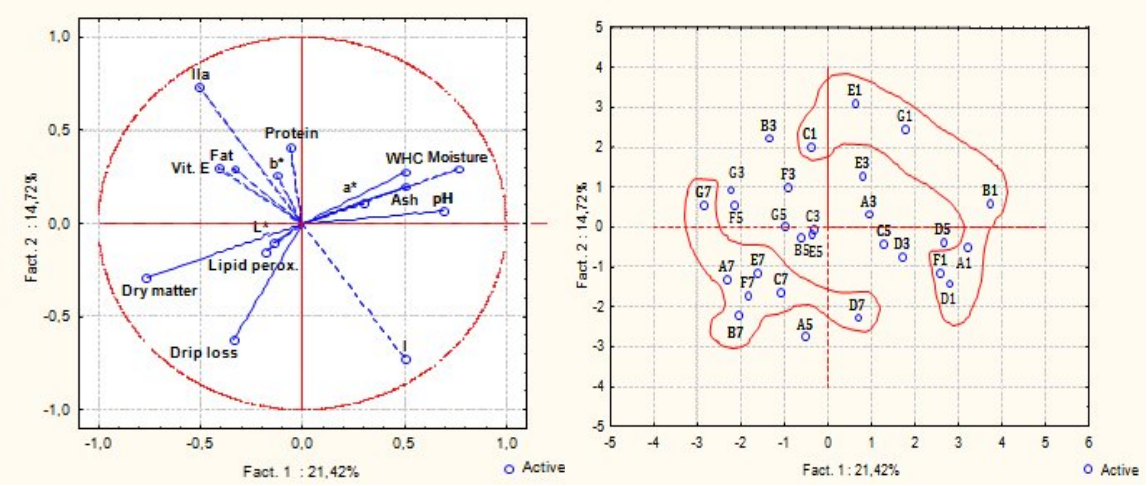


Figure 1. Plot for the variables (1a) and for the animals (1b) in the multivariate space following a principal component analysis (PCA). *In Figure 2a: WHC= Water holding capacity, I = MyHC I, IIa = MyHC II. In Figure 1b: A, B, C, D, E, F and G= Distribution of animals according to ageing group. 1, 2, 3…7=Days of ageing.*

Acknowledgement

The present work was achieved under the Sudanese-French program financed by the French embassy-Khartoum. The authors would like to thanks also the technicians at Herbivore Research Unit (INRA-Theix, France) and CIRAD (Campus International de Baillarguet, Montpellier), France for their assistance.

References

Agarwal, R. and S. D. Chase. 2002. Rapid, fluorimetric-liquid chromatographic determination of malondialdehyde in biological samples. J. Chrom. B: Anal. Tech. Biomed. Life Sci. 775:121–126.

Alderton, A. L., C. Faustman, D. C. Liebler and D. W. Hill. 2003. Introduction of redox instability of bovine myoglobin by adduction with 4-hydroxy-2-nonental. Biochemistry. 42:4398-4405.

AOAC, 2000. Association of Official Analytical Chemists. Official methods of analysis. Gaithersburg, MD, USA: AOAC International.

Arnold, R. N., K. K. Scheller, S. C. Arp, S. N. Williams and D. M. Schaefer. 1993. Dietary alpha-tocopheryle acetate enhances beef quality in Holstein and beef breed steers. J. Food Sci. 58:28-33.

Bell, E. F. 1987. History of vitamin E in infant nutrition. Am. J. Clin. Nutr. 46(1S):183–186.

Bonnet, M., A. Ouali and J. Kopp. 1992. Beef muscle osmotic pressure as assessed by differential scanning calorimetry (DSC). J. Food Sci. Technol. 27:399-408.

Botsoglou, N. A., D. J. Fletouris, G. E. Papageorgiou, V. N. Vassilopoulos, A. J. Mantis and A. G. Trakatellis. 1994. Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid-peroxidation in animal tissue, food, and feedstuff samples. J. Agric. Food Chem. 42:1931–1937.

Calkins, C. R., T. R. Dutson, G. C. Smith, Z. L. Carpenter and G. W. Davis. 1981. Relationship of fiber type composition to marbling and tenderness of bovine muscle. J. Food Sci. 46:708–710.

Campo, M. M., P. Santolaria, C. San˜udo, J. Lepetit, J. L. Olleta, B. Panea and P. Alberti. 2000. Assessment of breed type and ageing time effects on beef meat quality using two different texture devices. Meat Sci. 55:371–378.

Dransfield, E., J. F. Martin, D. Bauchart, S. Abouelkaram, J. Lepetit and J. Culioli. 2003. Meat quality and composition of three muscles

from French cull cows and young bulls. Anim. Sci. 76:387–399.

Durand, D., I. Savary-Auzeloux, I. Ortigues-Marty, T. E. Thomas, V. Scislowski, A. Peyron and D. Bauchart. 2006. Effet de la conservation de la viande sur les processus de peroxydations lipidiques et protéiques. In Proceedings of the 11th Journées de la Science du Muscle et de la Technologie de la Viande (pp. 77–78). 4th and 5th October, Clermont-Ferrand.

Durand, D., E. Thomas, A. Peyron, D. Gruffat and D. Bauchart. 2007. Influence of maturation and cooking treatments on lipid peroxidation and antioxidant status of bovine meat. In the proceedings of the 7th International Symposium on the Nutrition of Herbivores (Herbivores Nutrition for the Development of Efficient, Safe and Sustainable Livestock Production), 17-22 Sept. Beijing, China.

Faustman, C. and R. G. Cassens. 1990. The biochemical basis for discoloration in fresh meat: A review. J. Muscle Foods 1:217.

Faustman, C. and K. Wang. 2000. Potential mechanisms by which vitamin E improves oxidative stability of myoglobin. In: E. Decker and C. Faustman (Eds.), pp.135–152. Antioxidants in Muscle Foods. Chichester: John Wiley & Sons.

Favier, A. 1997. Oxidative stress: value of its demonstration in medical biology and problems posed by the choice of a marker. Ann. Biol. Clin. (Paris, France) 55:9-16.

Food and Nutrition Board, 2000. Dietary Reference Intakes: Applications in Dietary Assessment. Institute of Medicine. Washington, DC: National Academy Press. p.289.

Gobert, M., D. Gruffat, M. Habeanu, E. Parfait, D. Bauchart and D. Durand. 2010. Plant extracts combined with vitamin E in PUFA-rich diets of cull cows protect processed beef against lipid oxidation. Meat Sci. 85:676-683.

Hamm, R. 1986. Functional properties of the myofibrillar system and their measurements. In P. J. Bechtel (Ed.). pp.135–199. Muscle as food. New York: Academic Press, Inc.

Herrera, E. and C. Barbas. 2001. Vitamin E: action, metabolism and perspectives. J. Physiol. Biochem. 57(2):43–56.

Ho, C. Y., M. H. Stromer and R. M. Robson. 1996. Effect of electrical stimulation on postmortem titin, nebulin, desmin, and troponin-T degradation and ultra-structural changes in bovine longissimus muscle. J. Anima. Sci. 74:1563–1575.

Hocquette, J. F., P. Bas, D. Bauchart, M. Vermorel and Y. Geay. 1999. Fat partitioning and biochemical characteristics of fatty tissues in relation to plasma metabolites and hormones in normal and double-muscled young growing bulls. Comparative Biochemistry and Physiology A, 122, 127-138. Erratum, 1999. Comparative Biochemistry and Physiology A. 123:311-312.

Honikel, K. O. 2004. Water-holding capacity of meat. In: M. F. Pas, M. E. Everts and H. P. Haagsman (Eds.), pp. 389–400. Muscle development of livestock animals: Physiology, genetics and meat quality. Cambridge, MA: CABI Publishing.

Honikel, K. O., C. J. Kim, R. Hamm and P. Roncales. 1986. Sarcomere shortening of prerigor muscles and its influence on drip loss. Meat Sci. 16:267–282.

Hwang, I. L., C. E. Devine and D. L. Hopkins. 2003. The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness. Meat Sci. 65:677-691.

Immonen, K. and E. Puolanne. 2000. Variation of residual glycogen glucose concentration at ultimate pH values below 5.75. Meat Sci. 55(3):279–283.

Jakobsen, M. and G. Bertelsen. 2000. Color stability and lipid oxidation of fresh beef. Development of a response service model for prediction of the effect of temperature, storage time and modified attosphere condition. Meat Sci. 54:49-57.

Jobson, J. D. 1992. Applied multivariate data analysis. Volume II: Categorical and Multivariate Methods. Springer-Verlag, New York.

Jurie, C., I. Cassar-Malek, M. Bonnet, C. Leroux, D. Bauchart, P. Boulesteix, D. W. Pethick and J.F. Hocquette. 2007. Adipocyte fatty acid-binding protein and mitochondrial enzyme activities in muscles as relevant indicators of marbling in cattle. J. Anim. Sci. 85:2660-2669.

Jurie, C., D. Bauchart, J. Culioli, E. Dransfield, R. Jailler, J. Lepetit, A. Listrat, J. F. Martin, A. Ouali, Y. Geay and B. Picard. 2002. Modifications des caractéristiques du muscle Longissimus Thoracis chez les taurillons entre 15 et 24 mois. Rencontres, Recherche, Ruminants 9:265.

Kadim, I. T., Y. Al-Hosni, O. Mahgoub, W. Al-Marzooqi, S. K. Khalaf, R. S. Al-Maqbaly, S. S. H. Al-Sinawi and S. Al-Amri. 2009b. Effect of low voltage electrical stimulation on biochemical and quality characteristics of Longissimus thoracis muscle from one-humped Camel (*Camelus dromedaries*). Meat Sci. 82:77-85.

Kadim, I. T., O. Mahgoub, W. Al-Marzooqi, R. S. Al-Maqbali and S. M. Al-Lawati. 2008. The influence of seasonal temperatures on meat quality characteristics of hot-boned, m. psoas major and minor from goats and sheep. Meat Sci. 80:210–215.

Kadim, I. T., O. Mahgoub, W. Al-Marzooqi, S. K. Khalf, M. H. Mansour, S. S. H. Al-Sinani and I. S. Al-Amri. 2009a. Effects of electrical stimulation on histochemical muscle fibre staining, quality and composition of camel and cattle longissimus thoracis muscles. J. Food Sci. 74(1):S44-S52.

Kamal-Eldin, A. and N. Yanishlieva. 2002. N-3 fatty acids for human nutrition: stability consideration. Eur. J. Lipid Sci. Technol. 104:825-836.

Kassem, M. T., M. T. El-Sayed and A. Ahmed. 2004. Micostructural characteristics of Arabian camel meat. J. Camel Sci. 1:86-95.

Kerry, J. P., D. J. Buckley and P. A. Morrissey. 2000. Improvement of oxidative stability of beef and lamb with vitamin E. In: E. Decker and C. Faustman (Eds.). pp.229–261. Antioxidants in Muscle Foods. Chichester: John Wiley & Sons.

Lawrie, R. A. 1979. Meat Science, 3rd Ed., Pergamon Press, Oxford.

Marsh, B. B., T. P. Ringkob, R. L. Russel, D. R. Swartz, and L. A. Pagel. 1988. Recip. Meat Conf. Proc. 41:113.

Minamoto, S., K. Kanazawa, H. Ashida, G. Danno and M. Natake. 1985. The induction of lipid peroxidation in rat liver by oral intake of 9-oxononanoic acid contained in autoxidized linoleic acid. Agric. Food Chem. 49:2747–2751.

Offer, G. and P. Knight. 1989. Developments in Meat Science. In: R. Lawrie (Ed.). pp.14, Elsevier Science Publications, New York.

Offer, G. and P. Knight. 1988a. The structural basis of water-holding capacity in meat. Part 1: general principles and water uptake in meat processing. In: R. Lawrie (Ed.). pp.4:61–171. Developments in Meat Science, Developments in Meat Science, Elsevier Science Publications, New York.

Offer, G. and P. Knight. 1988b. The structural basis of water-holding capacity in meat. Part 2: drip losses. In: R. Lawrie (Ed.). pp.4:173–243. Developments in Meat Science, Elsevier Science Publications, New York.

Ouali, A. 1991. Animal Biotechnology and the Quality of Meat Production, L. O. Fiems, B. G. Cottin and D. I. Demeyer (Eds.). p.85. Elsevier Science Publications, Amsterdam.

Oury, M. P., R. Dumont, C. Jurie, J. F. Hocquette and B. Picard. 2010. Specificities of fibre composition and metabolism of the rectus abdominis muscle of bovine Charolais cattle. BMC Biochem. 11:12.

Picard, B. and I. Cassar-Malek. 2009. Evidence for expression of IIb myosin heavy chain isoform in some skeletal muscles of Blonde d'Aquitaine bulls. Meat Sci. 82:30-36.

Puolanne, E. and M. Halonenm. 2010. Review: Theoretical aspects of water-holding in meat. Meat Sci. 86:151-165.

Sasaki, K., M. Mitsuru and K. Kenji. 2001. Relationship between lipid peroxidation and fat content in Japanese Black beef Longissimus muscle during storage. Meat Sci. 59:407–410.

Savage, A. W. J., P. D. Warriss and P. D. Jolley. 1990. The amount and composition of the proteins in drip from stored pig meat. Meat Sci. 27:289-303.

Scislowski, V., D. Bauchart, D. Gruffat, P. M. Laplaud and D. Durand. 2005. Effects of dietary n -6 or n-3 polyunsaturated fatty acids protected or not against ruminal hydrogenation on plasma lipids and their susceptibility to peroxidation in fattening steers. J. Anim. Sci. 83:2162–2174.

Skidmore, J. A. 2005. Reproduction in dromedary camels: An update. Anim. Repr. 2:161–171.

Soltanizaheh, N., M. Kadivar, J. Keramat. and M. Fazilati. 2008. Comparison of fresh beef and camel meat proteolysis during cold storage. Meat Sci. 80:892-895.

Statistical Analysis Systems Institute Inc. 2001. SAS user's guide: statistics, version 8.2. Cary, NC, USA: SAS Institute Inc.

Talmadge, R. J. and R. R. Roy. 1993. Electrophoretic separation of rat skeletal muscle myosin heavy-chain isoforms. The Am. Physiol. Soc. 75:2337-2340.

Traber, M. G. and J. Atkinson. 2007. Vitamin E, antioxidant and nothing more. Free Rad. Biol. Med. 43(1):4–15.

Wegner, J., B. E. Albrecht, I. Friedler, F. Teuschner, J. H. Papstein and K. Ender. 2000. Growth and breed-related changes of muscle fiber characteristics in cattle. J. Anim. Sci. 78:1485–1496.