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Full Length Research Paper

Timber species from Afram arm of the Volta Lake in Ghana: Planing and sanding properties

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Volumes of timber logs (dead trees) in the Volta Lake in Ghana, that had become a dead trap for water transportation, were being extracted for utilization to increase the timber resource base. Unfortunately, their properties were not known for efficient promotion and utilization. The influence and relative significance of machine surface planing and sanding parameters in the production of good quality timber surface finish on four underwater timber species from the Volta Lake were investigated. The preparation of test specimens, testing, evaluation and grading of the tests specimens were conducted according to ASTM-D 143, 1666-87 and DIN 4768. A cutting depth of 2 mm was made constant. Wood specimens were planed and sanded at 12% moisture content and the surface qualities obtained were visually graded. Surface quality performance increased with decreasing rake angle and feed speed. Low rake angle of 15° with 6 m/min and 9 m/min feed speeds resulted in a high planing quality. The degree of magnitude of the chipped/torn grain defects decreased with decreasing rake angle and feed speed. Grit sizes of sand paper had significant effect on the surface quality of the four species. The chipped/torn grain defects observed were eliminated from all the wood species with grit size 40. High surface sanding quality was registered for all the species with grit 150.

Key words: Feed speed, planing quality, rake angle, sanding quality, surface quality.

INTRODUCTION

The flooding of the Volta Lake in Ghana after its creation in 1965 submerged thousands of hardwood trees (Figure 1), which have been posing danger to water transportation, but at the same time could be useful as timber when harvested. As Ghana's timber resource base is dwindling, there is the need to find alternative resources to feed the timber industry, hence attention being turned to the large volumes of timber under the Volta Lake. However, like the lesser-used and lesser-

known timber species, the underwater species need to be promoted to enhance their utilization, but it is not known whether their properties remain the same as the species from the natural forest. It is therefore necessary to establish their properties, which include machining, for their efficient promotion. The study was to determine the planing and sanding characteristics of four timber species harvested from the Volta Lake and recommend some potential uses. Some of the timber logs that had been

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Figure 1. Some hardwood trees in the Volta Lake.
Source: Field (2007).

harvested and identified as *Manilkara multinervis* (Berekankum), *Erythrophyleum guinense* (Potrodom), *Diospyros mespiliformis* (Savannah Ebony) and *Cylicodiscus gabunensis* (Denya / Okan) with their scientific and local names respectively, were used for the study.

The results from the study will facilitate the promotion of the new timber resource, thereby making more timber logs available for processing and utilization by the timber industry and revive some of the timber companies that are at the point of collapse. This will lead to reduction of the rate of accident on the Volta Lake, generation of employment which will enhance the livelihood of the local communities around the Volta Lake, save the Ghanaian forests from total depletion in the near future and increase in the country's annual revenue through export of timber products.

One of the most important advantages of wood is its easy machinability in contrast to metal and plastic products (Sofuoğlu and Kurtoğlu, 2014). Karpat and Özel (2007) have indicated that for manufacturers to remain competitive on both local and international markets, they must increase their productivity while maintaining, if not improving, product quality. Any surface defects due to an improper machining process will also reduce the quality of the final product, resulting in an increase in the cost of the manufactured unit (Malakoçoğlu and Özdemir, 2006).

In the machining operations, production rate, cost, and product quality are three incompatible objectives (Cus and Zuperl, 2006). They continue that as the machining industry welcomes the introduction of new materials and cutting tools, it finds itself undergoing a rapid development which is giving rise to processes of highly complex and non-linear phenomena.

Ambrogio et al. (2008) and Tamizharasan et al. (2006) have stated that surface quality in machining is an essential consumer requirement because of its impact on product performance. Also the characteristics of machined surfaces that significantly influence its physical properties have been pointed out by Basheer et al. (2008). Sogutlu and Togay (2011) have also stated that surface roughness is an important factor in the quality of the internal and external decorative elements produced from wood. Chang and Lu (2006) and Oktem et al. (2006) have indicated that surface roughness plays an important role in determining the quality of a machined product.

Surface roughness has been defined by Benardos and Vosniakos (2003) as the superimposition of deviations from a nominal surface from the third to the sixth order where the orders of deviation are defined by international standards, DIN 4760 (1982). Correa et al. (2009) have also defined surface roughness as the functional behavior of a part. Roughness is a measure of the fine irregularities on a surface. The shape and dimensions of

the irregularities on a surface determine surface quality of a product. According to Hiziroglu and Kosonkorn (2006) the surface roughness of a wood panel plays an important role since any surface irregularities may show through thin overlays reducing the final quality of the wood panel. Roughness is thus an indicator of process performance and must be controlled within suitable limits for particular machining operations and that the factors leading to roughness formation are complex (Pal and Chakraborty, 2005). Benardos and Vosniakos (2002) and Tan et al. (2012) have declared that statistically significant in roughness formation are the absolute values of cutting parameters such as depth of cut, feed speed, and components of cutting force.

Various techniques, according to Gurau et al. (2005); Hendarto et al. (2005) and Sandak and Tanaka (2005) that have been used to determine surface roughness include optical, pneumatic, electrical, ultrasonic, photographic and stylus methods. It has also been found by Sieminski and Skarzynska (1989) that the stylus-type profilometer was suitable within the tested methods for the measurement of the surface roughness of wood.

Surface roughness is influenced by the rays, pore sizes, knots and reaction wood (Taylor et al., 1999). Skaljic et al. (2009) have also reported that physical and mechanical properties and anatomical structure of wood affect the surface roughness. As the number of blades increases, the surface roughness values decrease, and as the feed rate increases, the surface roughness also increases (Ors and Baykan, 1999; Roger and Cool, 2008).

In planing, the different cutting angles and number of cutters had different effects on surface quality in terms of wood species (Ratnasingam and Scholz, 2007; Malkoçoğlu and Özdemir, 2006). For good machining quality, the cutters used in the machining of the material should be sharpened and machinery should be maintained and stabilized (Sofuoğlu and Kurtoğlu, 2014).

In general, better machining performance is obtained with decreasing feed speed (Skaljic et al., 2009). Moreover, increasing feed speed has been reported to cause strong machining defects (Malkoçoğlu, 2007). Farrokhpayam et al. (2010) have also stated that small differences between the different levels of the feed rate affect surface quality and that the slowest feed rate of 8 m min⁻¹ gives the best results, with progressively poorer work as fastest feed speed is used. Fujiwara et al. (2001) have confirmed in various studies that if variables such as the feed rate are not selected correctly, then the desired surface quality will not be obtained. Sogutlu and Togay (2011) have reported that when the Oriental beech (*Fagus orientalis* Lipsky) and Scotch pine (*Pinus silvestris* L.) wood specimens were prepared by planing, the surface roughness was less in the tangential cut as compared to the radial cut. Furthermore, the surface roughness was less in planing with 4 blades as compared to planing with 2 blades. Best surface quality could be

achieved where the depth of the final cut is from 1 to 2 mm and that excess stock removal of more than 2 mm will cause chipped/torn grain or pickup defects (Tjernlund, 1984).

In planing and sanding, the degree of surface roughness is dependent on the properties of the raw material and production processes (Hendarto et al., 2005; Nemli et al., 2005, 2007). A study by Nemli et al. (2007) shows that grit sizes, feed speed of panels and the feed power of the heads of the sander are the main considerations for a successful sanding operation. Also a report by Beaty (1983) and NPA (1993) indicate that sanding equipment, coated abrasive belt specifications, production scheduling and correct machine setup are the main considerations for successful sanding operation. Sanding is the most common and most influential operation for achieving surface quality during the phase of surface preparation (Skaljic et al., 2009). There is the need to recognize and understand the key factors and variables that affect selection of sanding system, sanding equipment, sanding quality and the ultimate performance of the finished product.

The study sought to investigate the effect of the process parameters that exert significant influence on the surface quality of Savanna Ebony, Berekankum, Denya/Okan and Potrodom (extracted from the Volta Lake in Ghana) after planing and sanding operations. Specifically, the effects of the rake/hook angles, spindle speeds, feed speeds and grit sizes of sand paper on wood planing and sanding qualities were investigated.

MATERIALS AND METHODS

Collection of samples

Four (4) timber species used for the study were *Manilkara multinervis* (Berekankum), *Erythrophyleum guinense* (Potrodom), *Diospyros mespilliformis* (Savannah Ebony) and *Cylicodiscus gabunensis* (Denya / Okan) in their scientific and local names respectively. These were the timber species that had been harvested from the Volta Lake by Clark Sustainable Resource Development (CSR) at the time of the study. According to their utilization status, *M. multinervis* was a lesser-known timber species while the remaining three were lesser-used timber species (Oteng-Amoako, 2006). Using through and through sawing pattern, the logs were milled into lumber at Forestry Research Institute of Ghana (FORIG) under the Council for Scientific and Industrial Research (CSIR), Fumesua near Kumasi in the Ashanti region. The lumber pieces obtained from each timber species were stacked for air drying.

Preparation of tests specimens

ASTM D 143-94, ASTM D 1666-87 (ASTM, 2004; 2007) and DIN 4768 with some required adjustments were used to prepare the planing and sanding tests specimens. Some lumber boards of 25 mm thickness were taken from the stacked lumber, kiln dried to 10% moisture content for the preparation of 300 specimens of dimensions 25 × 100 × 900 mm. They were then conditioned to 12% equilibrium moisture content at 27°C and 65% relative humidity

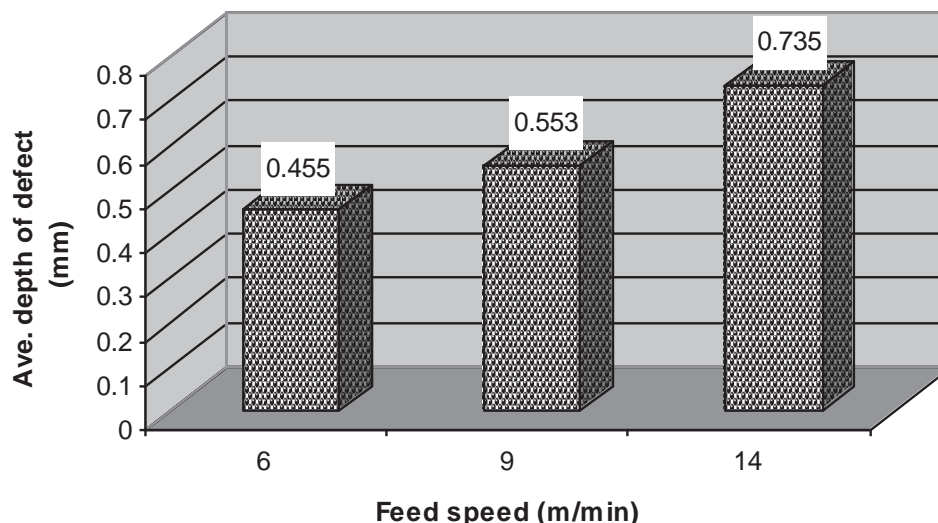


Figure 2. Average depth of chipped/torn grain defect with two cutting angles.

for ten days before the planing test.

Planing test

Planing was done using a combined surfacing and thickening machine of type 610 mm × 230 mm 'D.A.A' at a spindle speed of 5,200 rpm. A cutter head with a pair of planer knives were used. The selected feed speeds and rake/hook angles were 6 m/min, 9 m/min and 14m/min and 15° and 20° respectively. With six planing operations, 25 test specimens per operation were made to pass through the planer until all the operations were completed before they were graded in accordance with ASTM D 1666-87. A constant depth of cut of 2 mm per face of two for each specimen was set.

Grading

The specimens were then examined visually, with the aid of a hand lens, evaluated and classified, according to ASTM D 1666-87, on the basis of five quality grades [grade 1 = Excellent or defect-free; grade 2 = good; grade 3 = fair; grade 4 = poor and grade 5 = rejected or very poor. (Grades 2-5 were dependent on the degree of the defect observed)]. Percentage defect-free specimens was calculated for each rake angle and feed speed, as shown in Table 1. The depth of each chipped/torn grain (which is a machining defect), observed on the surfaces of the specimens, was measured and the average for each operation (a rake angle and feed speed) estimated (Table 2).

Sanding test

Sanding operation was undertaken with a vertical belt sander of model CL300. Five grit sizes of sand paper, P40, P60, P100, P120 and P150 were used. The grits were with open-coat paper-backed aluminum oxide sandpapers coated with anti-static zinc stearate. Sanding was carried out along the grain. The defective specimens (chipped/torn grain) were selected from the planed specimens and sanded with grit sizes 40 and 60 to assess the possible elimination of the defect. The specimens without the defects were sanded with

grits 100, 120 and 150 for the assessment of the final surface quality for the application of finishes. The degree of scratches observed on all the specimens per species with grits 100, 120 and 150 were noted. The same grading procedure as stated above was used to grade the specimens and the percentage defect-free specimens (grade 1) were estimated for both sanding activities per species (Tables 3 and 4).

RESULTS AND DISCUSSION

Planing properties

The results on planing properties were based on clear specimens (ASTM D 143-94 and 1666-87, 1994). To identify the best planing performance, each planing condition was examined separately. Table 1 shows the percentage defect-free specimens with respect to feed speeds and or rake/hook angles for the four timber species. Analysis of variance (ANOVA) was used to test for the significant differences among the three feed speeds, two rake angles and the species. At $P = 0.05$, the significant differences in planing quality existed among the different feed speeds. The percentage defect-free specimens increased with decreasing feed speed. This means that the lower the feed speed the better the surface quality. This was consistent with all the timber species as feed speed changed from 6 m/min to 14 m/min (Table 1). This is also reflected in Table 2 and Figure 2 where the average depth of chipped/torn grain generated after planing decreased with decreasing feed speed. Therefore the best surface quality was generated with a feed speed of 6 m/min (72-100%) followed by 9 m/min (68-92%) and then 14 m/min (44-72%). Figure 3 shows the average percentage defect-free specimens for

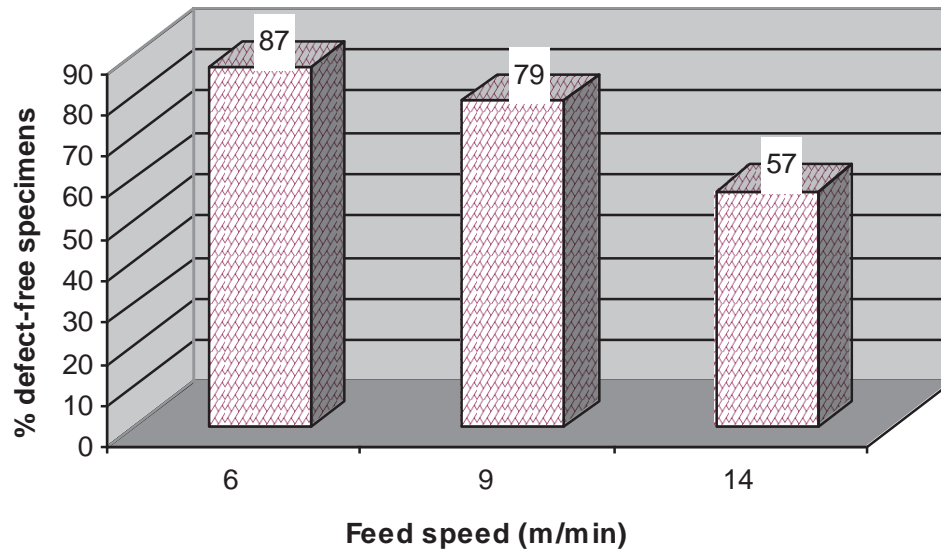


Figure 3. Average percentage defect-free specimens for two rake angles.

the two rake angles. The trend is in agreement with Ors and Baykan (1999) and Roger and Cool (2008) whose reports indicate that as the feed speed increases, the surface roughness also increases. It also confirms the statement by Fujiwara (2001) that if variables such as feed speed are not selected correctly, the desired surface quality will not be obtained. Results of the average percentage defect-free specimens indicate that feed speeds 6, 9 and 14 m/min could be rated as high, medium and low surface quality respectively.

The surface quality of all the timber species was affected by the rake angle, as shown in Table 1. The results indicate that the percentage defect-free specimens increased with decreasing rake angle and that 15° rake angle recorded higher percentages than with 20°. The average percentages of defect-free specimens estimated for 15 and 20° rake angles are 81% (high surface quality) and 67% (medium surface quality) respectively. This implies that surface quality was higher with the lower rake angle of 15° than with 20°. The results showed significant differences between the rake angles at $P=0.05$. A similar trend/pattern has been recorded by Fortin (2001) that “the proportion of defect-free pieces increased as the rake angle decreased from 20 to 15°” and therefore has proposed a rake angle of 15° for planing White spruce wood from plantation forests. Gilmore and Barefoot (1974) have also proposed 15° rake angle for planing some tropical wood species from South America. From Table 2, as the rake angle decreased from 20 to 15° (for each feed speed) the depth of the chipped/torn grain defect also decreased in magnitude. For example, the average depth of the defect at 15° hook angle varied from 0.49 mm (for Ebony) to 0.56 mm (for Potrodom) while that at 20° also varied from 0.57 mm (for Ebony) to 0.65 mm (for Potrodom). The

minimum and maximum average magnitudes of the depth of the defect at 15 and 20° with the three feed speeds were estimated to be 0.36-0.73 and 0.44-0.8 mm respectively. On the average 0.6 mm maximum depth was estimated for every species. Therefore the average magnitude of the defect for the three feed speeds was lower with 15° rake angle than 20° as shown in Figure 4.

Generally the planing results have shown that feed speed and rake angle are among the major factors that control wood failure during planing as have been recorded by Sogutlu and Togay (2011) and Fortin (2001). Hence feed speeds of 6, 9 and 14 m/min with a rake angle of 15° will be more appropriate for the planing of the selected timber species.

Comparing species in terms of surface quality, the average percentage defect-free specimens for all the conditions ranged between 67% (for Berekankum) and 80% (for Savanna Ebony). At 95% confidence interval, there was no significant difference between the surface quality of Denya/Okan and Potrodom which recorded an average of 74 and 75% defect-free specimens respectively. This means that the two timber species may have some common anatomical characteristics; hence planing them with the same machine setting will generate equal surface planing quality. According to Oteng-Amoako (2006), Denya and Potrodom are both coarse textured species and therefore confirms the findings of Tailor et al. (1999) that surface roughness is also influenced by rays and pore sizes. Figure 5 shows grade 1 planed specimens of Ebony, Denya, Berekankum and Potrodom. Introducing performance rate classification whereby 80-100% = high surface quality; 60-9% = medium surface quality; 40-59% = low surface quality and below 40% = poor surface quality, Savanna Ebony, on the average, recorded a high surface quality while the

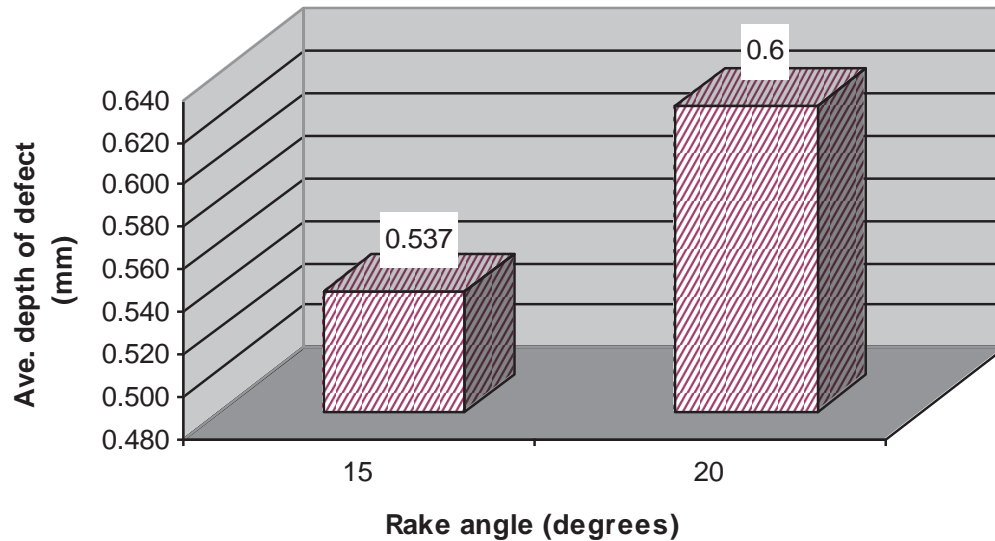


Figure 4. Average depth of chipped/torn grain defect with three feed speeds.



Figure 5. Grade 1 specimens of the four timber species.

three other species attained medium surface quality (Table 1). The average percentage surface quality for all the species with the planing tests conditions was estimated to be 74.2%, which from the machining rating introduced, could be classified as medium surface quality for every species. A report by Farmer (1972) shows that

a rake angle of 10° is required for satisfactory planing of Denya (from the natural forest) when it is quarter-sawn, but the feed speed(s) and the percentage defect-free specimens were not specified. Denya (from underwater) with an average score of 75% defect-free specimens (medium surface quality rating) may be equivalent in

Table 1. Percentage defect-free specimens at two hook angles with three feed speeds

Species	Percentage defect-free specimens						Average on species basis
	6 m/min		9 m/min		14 m/min		
	15°	20°	15°	20°	15°	20°	
Berekankum (<i>Manilkara multinervis</i>)	84	72	76	68	60	44	67.3
Potrodom (<i>Erythrophyleum guinense</i>)	92	84	88	68	64	48	74.0
Savannah Ebony (<i>Diospyros mespiliformis</i>)	100	88	92	76	72	52	80.0
Denya / Okan (<i>Cylicodiscus gabunensis</i>)	92	84	88	72	64	52	75.3
Average % per species	92	82	86	71	65	49	74.2

Table 2. Average depth of chipped/torn grain machining defect generated on each species with the planing conditions studied.

Species	Magnitude of defect in millimeters (depth)						Average on species basis
	6 m/min		9 m/min		14 m/min		
	15°	20°	15°	20°	15°	20°	
Berekankum (<i>M. multinervis</i>)	0.42	0.50	0.50	0.62	0.71	0.78	0.6
Potrodom (<i>E. guinense</i>)	0.43	0.52	0.52	0.65	0.73	0.79	0.6
Savannah Ebony (<i>D. mespiliformis</i>)	0.36	0.44	0.47	0.55	0.65	0.72	0.5
Denya / Okan (<i>C. gabunensis</i>)	0.44	0.53	0.51	0.60	0.70	0.80	0.6
Average % per species	0.41	0.50	0.50	0.61	0.70	0.77	0.6

Table 3. Percentage defect-free specimen after sanding to remove defects with two different grit sizes.

Species	Percentage defect-free specimen	
	Grit size P60	Grit size P40
Berekankum (<i>M. multinervis</i>)	80	100
Potrodom (<i>E. guinense</i>)	80	100
Savannah Ebony (<i>D. mespiliformis</i>)	100	100
Denya / Okan (<i>C. gabunensis</i>)	80	100

Table 4. Scratching tendencies of specimen after sanding with three different grit sizes.

Species	Percentage scratching tendency		
	Grit size P100	Grit size P120	Grit size P150
Berekankum (<i>M. multinervis</i>)	56	72	80
Potrodom (<i>E. guinense</i>)	60	76	88
Savannah Ebony (<i>D. mespiliformis</i>)	48	60	80
Denya / Okan (<i>C. gabunensis</i>)	60	80	92

planing quality to that from the natural forest.

Sanding properties

Table 3 shows the percentage defect-free sanded specimens when sand paper of grit sizes 60 and 40 were

used in that order. At $P = 0.05$, there were significant differences between grit sizes of sand papers used. With the chipped/torn grain defects that were generated after planing, grit size 60 was able to remove them from Savannah Ebony, hence scoring 100%; while Berekankum, Potrodom and Denya with 80% defect-free specimens, still had some degree of the defects on the

surfaces of some of the specimens. Using sandpaper with grit size 40, the entire chipped/torn grain defects were eliminated from the wood specimens. This implies that sand paper with grit size 40 is capable of eliminating any chipped/torn grain defect from the wood members of the four timber species before further sanding could be under taken for the application of finishes. It was therefore established that the various degree of magnitude of the defect that was eliminated with grit 60 were comparatively shallower and did not affect the thickness of the specimens involved. Such shallower defect was classified by the study as chipped grain while torn grain was referred to that defect which reduced the thickness of the wood specimens above 1.5 mm. Such a reduction could affect the service life of a dimensioned wood member that is put in service (for instance, in production of furniture or roofing). According to Owusu et al. (2011), the sanding quality of Denya (from the natural forest) with grit P60 at 12% moisture content was rated as high, which is comparable to the results obtained in this study.

Grit sizes of 100, 120 and 150 were used to finally prepare the surfaces of the specimens for the application of finishes. This means that scratches formation on wood surfaces was possible at different degrees and hence has to be brought to the barest minimum. The degree of scratches generated on the wood surfaces was observed to decrease with increasing grit size and that the degree of scratches was minimal with grit size 150 than 120 and 100. Therefore higher grit size (in this case, 150) of sand paper is recommended for better finishing in order to effectively add value to tertiary wood products. This shows that grit sizes of sand paper affect surface quality and hence supports the research findings observed by Nemli et al. (2007). The scratches per grit size were observed to be prevalent on Savanna Ebony followed by Berekankum, Potrodom and Denya/Okan. This could be attributed to the texture of the species, and that scratches were obvious on fine textured species than course textured. Therefore the statement by Hendarto *et al.*, (2005); Nemli *et al.*, (2007) and Nemli *et al.*, (2005) that the degree of roughness during sanding, as machining activity, is dependent on the properties of the raw material and the production processes is confirmed. It also supports the earlier finding by Owusu et al. (2011) that “the finer the texture of the species, the more pronounced the scratching tendency”. The result on the scratching tendency of Denya (from underwater) with grit P120 is equivalent to Denya (from natural forest) at 12% moisture content (Owusu et al., 2011).

Potential uses of the species

Based on the results achieved, the following potential uses are proposed for the four timber species extracted from the Volta Lake: Berekankum (*M. multinervis*):

Furniture products and could be used in replace of African Mahogany. Savannah Ebony (*D.mespiliformis*): Furniture products and walking sticks and could be a good substitute for *M. altissima*. Potrodom (*E. guinense*) and Denya / Okan (*Cylicodiscus gabunensis*): Interior carpentry and walking sticks

Conclusions

The study has revealed that the four timber species harvested from the Volta Lake have good planing and sanding performances. The surface planing quality increased with decreasing rake angle and feed speed. The best planing condition obtained was 15° rake angle with feed speeds of 6m/min and 9 m/min. The machining defects observed were chipped and torn grain. The average range of magnitudes of the defect in terms of depth with 6, 9 and 14 m/min feed speeds for the four species at 15 and 20° rake angles were 0.36-0.73 and 0.44-0.80 mm respectively. Shallower defect was classified by the study as chipped grain while torn grain was referred to that defect which reduced the thickness of the wood specimens above 1.5 mm. Consistently, Savanna Ebony performed better in all the planing operations.

Sand paper with grit size 40 was capable of eliminating any chipped/torn grain defect from the wood specimens of the four timber species. The degree of scratches generated on the wood surfaces decreased with increasing grit size and that was minimal with grit size 150 than 120 and 100. Therefore higher grit size (in this case, 150) is recommended for final sanding to achieve better finishing in order to effectively add value to tertiary wood products. Scratches were obvious on fine textured species than course textured.

The results obtained for Denya/Okan are comparable (if not better) to that from the literature, which come from the natural forest. Since the machining performance of Potrodom (*Erythrophyleum guinense*), Berekankum (*Manilkara multinervis*) and Savanna Ebony (*Diospyros mespiliformis*) are equivalent to Denya/Okan (*Cylicodiscus gabunensis*), it implies that their machining properties could also be comparable with those from the natural forest.

RECOMMENDATIONS

The results indicate that the four species from the Volta Lake recorded, at least, medium machining surface quality, hence a higher potential for processing and utilization. Wood users should therefore patronize them when seen on the timber market.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Effects of organic and chemical fertilizers on yield and total soluble solids (TSS) in Banana Cavendish group (AAA)

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Banana fruit production in the Sudan covers a wide spectrum of activities ranging from local utilization of fruit with self-sufficiency through small farming for local market to large plantation for export. It is one of the most important fruits of Sudan. Therefore, a field experiment was conducted at two locations during 2009\2010 and 2010\2011 in split-split plot layout with four replications. The main plots were the two varieties of Cavendish Dwarf and Grand Nain (AAA) and the sub-plots are the fertilizer treatments arranged randomly. The treatments consisted of urea (400 g/mat/year), NPK (200 g/mat/year) and in combination with organic fertilizers ,compost 10 kg/plant/year, manure 5 kg/plant/year). The results indicated that there were significant effects ($p \leq 0.05$) in fruit parameters (weight of bunch, hand, fingers and number of hands\bunch and fingers\bunch). The fertilizer treatment urea (400 g/mat/year), and urea plus organic manure in the two seasons were highly significant different. The results showed that there were highly significant increase in growth resulted from all fertilizer treatments and reflected in earlier shooting, 198 days in the main crop, 142 days in the first ratoon, and shorter time to flowering and harvest, 35 days in the main crop and 32 days in the first ratoon crop in Dwarf Cavendish compared to Grand Nain cultivars in the two seasons.

Key words: Banana, varieties, fertilizers.

INTRODUCTION

Banana is one of the oldest tropical fruits cultivated by man from pre-historic times (INRI, 2001). Banana (*Musa spp*) member of the Musaceae family is one of the most important tropical fruit crops in the world and is a major tropical food crop with annual world production of around 88 million tons (Sharrock et al., 2001). Dwarf Cavendish is the predominant cultivar grown in Sudan especially in the Blue Nile and Kassala States (Elhassan et al., 2006;

Elbashir and Imam, 2010). The experiment was done with two cultivars of Cavendish group, dwarf and giant Cavendish. Dwarf is the smallest or short and better adapted to a cool climate than any other cultivar. "Giant Cavendish" compared to dwarf is slightly taller and its French name is "Grand Nain", Samson (1980). 'Grand Nain is an outstanding banana variety growing from 180 to 240 cm (6 to 8 feet) tall and with solid green color. It is

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very attractive as landscaping potential. The 'Grand Nain cultivar produces very large heads of delicious fruits. Bunches may weigh up to 60 kg (150 Lbs.). This is a commercial variety that you may buy in the grocery store. The word "banana" is a general term embracing a number of species or hybrids in the genus *Musa* of the family Musaceae. Most edible banana fruits are seedless. It belongs to the Species (*Musa acuminata*). Banana can be divided into two main groups, one is the dessert bananas which constitute 43% of world production (Jones, 2000), the fruits of this group are chiefly eaten raw when ripe as a dessert fruit, and the other group is the wild one filled with a great number of very hard seeds with thickness of 3 to 5 mm. Banana fruit production in the Sudan covers a wide spectrum of activities ranging from local utilization of fruit, through small farming for local market, to large plantation for export. The organic manure which was made from compost provides the nutrients necessary for the crop as nitrogen, phosphorus, potassium etc., and improves the chemical and physical characteristics of the soil. The soil fertility increases and it becomes soft and porous. Thus humidity is also maintained.

In the Sudan commercial banana production is for local market. The cultivation is concentrated to the alluvial Delta of Gash River in Kassala State. In Kassala State there was a reduction in acreage. This reduction was mainly attributed to scarcity of irrigation water and to poor husbandry practices (ACCORD, 2010). There are many problems that hinder the production of banana such as nematode infestation, scarcity of suckers and the dependence on one variety, in addition to the lack of knowledge about the cultural practices, and absence of detailed agricultural operations, such as fertilization planting methods, variety, spacing, and pruning, ripening and marketing programs of the future. Therefore, to stimulate banana crop production and make it commercially feasible, considerable research should be geared towards investigating, recognizing and solving the problems facing banana industry in the country. Fertilization is an important and limiting factor for growth and productivity of banana. The plants remove large amounts of nutrients from the soil as it is well known that banana needs large amounts of fertilizers especially nitrogen. Therefore the major problems facing banana growers are the high costs of manufactured fertilizers needed for banana production (El Moniem et al., 2008; ACCORD, 2010). Besides, these chemical fertilizers are considered as air, soil and water polluting agents during their production and utilization. Consequently, it has drawn the attention of researchers and banana growers to use the organic fertilizers, which are safe for human, animal and environment, as a partial substitute for mineral source. Thus, it is preferred to use these natural fertilizers to avoid pollution and to reduce the costs of chemical fertilizers. In the experiments two types of fertilizers were used, organic manure (cow and chicken) and chemical fertilizer (Urea+ NPK) and the combinations of the two fertilizers.

The objective of this study is to investigate the effect of organic manure and chemical fertilizers on growth and yield of two banana cultivars namely Dwarf Cavendish and Grand Nain, and to determine the dose of fertilizers which influence the yield and fruit characteristics, like total bunch weight, middle hand weight and number of fingers per middle-hand.

MATERIALS AND METHODS

The present study was carried out in Alliab area in the River Nile State in Sudan, during the period from June to September (2009\2010 and 2010\2011). The climate of the area is desert, hot summer, warm winter and with summer rain (Van der Kevie, 1976). The soil samples were analyzed for physical and chemical characters according to the standard procedure (Table 1). The design was split plot design. The experiment was carried out as split-split plot based on randomized complete block design (RCBD) with four replications and 18 treatments; the main plots are the two varieties of Dwarf Cavendish (AAA) and Grand Nain (AAA) and the sub-plots are the fertilizer treatments arranged randomly. Planting materials are suckers of the banana cultivars, Dwarf Cavendish and Grand Nain, four months old, sword type. The suckers were obtained from a plantation in Shendi locality. The experiment in sub plots consisted of, urea 400 g/mat/year, NPK (18:18:18) : 18 g/mat/year, and the organic fertilizers, compost 10 kg/plant and manure 5 g/mat/year). The bunches were weighed using spring balance. To determinate the weight of fingers, the weight of all hands in a bunch was divided by the total number of fingers in that bunch.

RESULTS AND DISCUSSION

Generally the results in the two seasons indicated that there were significant differences ($p \leq 0.05$) in weight of bunch, number of hands/bunch, number of fingers/bunch, and weight of hands/bunch and earlier times to flowering and to harvesting in all fertilizers treatments, especially the treatments manure (M), Compost + Urea (C+U) and Urea (U) as compared to the control (Table 2). In addition to that, the cultivar Grand Nain was more responsive than Dwarf Cavendish in all parameters (Figure 1); this result indicates that cultivars respond differently to fertilizer treatments and this is may be due to the genetic differences between the two cultivars. These findings are in agreement with that of Gangwar and Niranjana (1990) who studied the effect of inorganic fertilizers and FYM on the rain-fed fodder sorghum. They found that addition of FYM+50% recommended doses of inorganic fertilizer resulted in significantly higher plant height, dry weight, and fresh weight and increased the uptake of N, P and K compared with the control. This is also in agreement with Singh and Suryanaryana (1999) and Manica et al. (1978) who recorded the highest bunch weight and fruit yield by 200 g N. Dawoud et al. (1991) studied the response of dwarf Cavendish banana to nitrogen fertilization on heavy clay soils. The heavy clay soils of Sudan are low in N and organic matter. The effect of N (0-276 g/mat/year on the growth and yield of bananas Cv. Dwarf Cavendish (plant

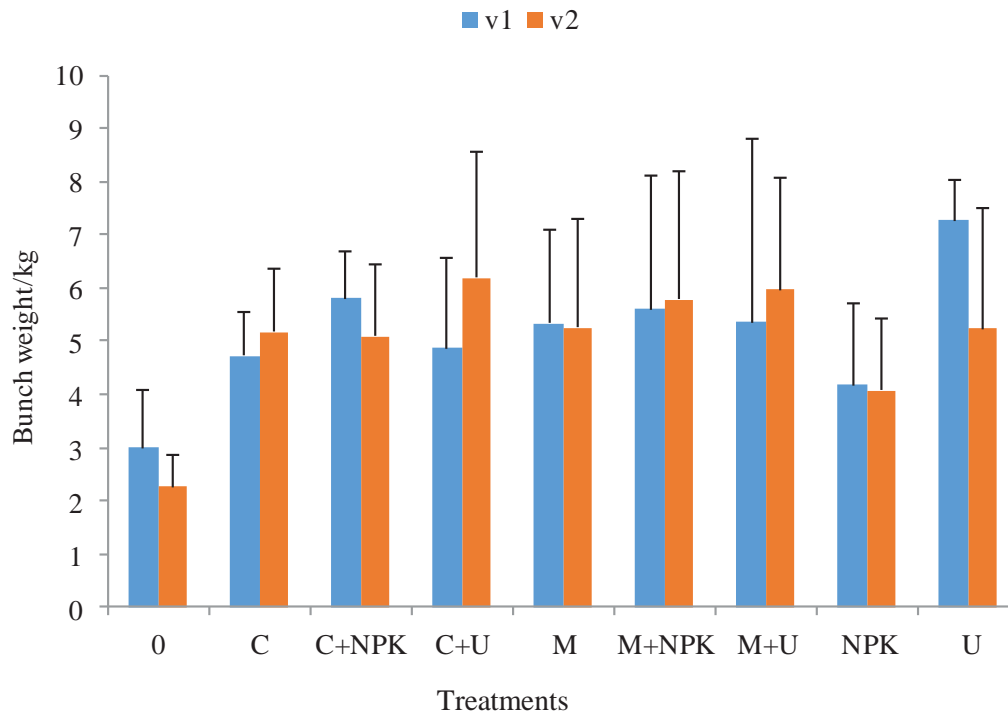


Figure 1. Bunch weight, kg/ha for Dwarf Cavendish plant crop. v 1= Dwarf Cvedish; v 2= Grand Nain. C= Compost, M= Manure, NPK= Inorganic fertilizer.

Table 1. Soil physical characteristics at the experiment site in Alliab area.

Type of soil	Sand	Clay	Silt
Percentage (%)	45	53	2

Table 2. Effect of fertilizers (organic and commercial) treatments on yield components of Dwarf Cavendish plant crop, season (2009/2010-2010/2011).

Treatment	Weight of bunch/kg	Weight of hand/ bunch/ kg	Number of hand/bunch/cm	Number of fingers/bunch
U	16.23 ^a	2.5 ^{ab}	6.3 ^{ab}	10.7 ^{abc}
NPK	14.0 ^{bc}	2.7 ^{ab}	5.1 ^{bc}	9.4 ^{bc}
M	15.3 ^{ab}	2.5 ^{ab}	6.0 ^{abc}	11.7 ^a
C	14.9 ^{ab}	2.4 ^{ab}	6.1 ^{ab}	10.4 ^{abc}
M+U	16.9 ^a	4.8 ^a	7.1 ^a	10.9 ^{ab}
M+NPK	15.6 ^{ab}	5.2 ^a	5.8 ^{abc}	10.0 ^{abc}
C+U	15.5 ^{ab}	4.6 ^a	6.4 ^{ab}	11.4 ^{ab}
C+NPK	15.5 ^{ab}	3.7 ^{ab}	6.1 ^{ab}	10.3 ^{abc}
C ₀	10.8 ^c	1.9 ^c	4.6 ^c	8.62 ^c
LSD	1842	1.79	1.3	1.87
SE+	594.9	0.58	0.448	0.655
CV%	34.5	43.6	21.6	17.5

*The same letter(s) were not significantly different according to Duncan's Multiple at Range test 5% level. M (manure), U (urea), and C (compost), NPK (inorganic fertilizer).

Table 3. Effect of fertilizers (organic and N application) treatments on yield components of Grand Nain plant crop seasons 2009/2010-2010/2011).

Treatment	Weight of bunch/kg	Weight of hand/bunch/kg	Number of hand/bunch/cm	Number of fingers/bunch
Urea	17.8 ^a	2.8 ^a	8.4 ^b	12.9 ^{ab}
NPK	16.9 ^a	2.7 ^a	7.3 ^{bc}	11.0 ^{ab}
Manure	16.4 ^a	2.7 ^a	7.8 ^{bc}	11.1 ^{ab}
Compost	16.2 ^a	2.6 ^a	8.0 ^b	12.6 ^{ab}
M + U	18.4 ^a	2.7 ^a	9.1 ^a	12.6 ^{ab}
M + NPK	16.1 ^a	2.8 ^a	7.6 ^{bc}	11.0 ^{ab}
C + U	17.2 ^a	2.8 ^a	8.0 ^{bc}	13.3 ^a
C + NPK	17.7 ^a	2.7 ^a	7.8 ^{bc}	11.6 ^{ab}
Control	12 ^b	1.7 ^b	6.5 ^c	10.1 ^b
LSD	3.07	1.88	1.48	2.0
SE+	1.08	0.66	0.52	0.70
CV%	43.83	29.89	18.47	16.89

*The same letter(s) were not significantly different according to Duncan's Multiple at Range test 5% level Key: M (manure), U (urea), and C (compost), NPK (inorganic fertilizer).

Table 4. Effect of fertilizers on TSS in different fruit stages (Green and Ripe) of Dwarf Cavendish.

Treatment	Green mature	Ripe fruit
Urea	6.2 ^{bc}	17.5 ^{ab}
NPK	6.4 ^{bc}	14.9 ^b
Manure	6.8 ^b	17.8 ^{ab}
Compost	6.5 ^{bc}	15.9 ^{ab}
M + U	8.2 ^{ab}	17.5 ^{ab}
M + NPK	9.3 ^a	20.3 ^a
C + U	6.7 ^{bc}	15.3 ^b
C + NPK	4.5 ^c	14.7 ^b
LSD	1.9	4.3
SE+	0.805	1.593
CV%	28.8	26.1

*The same letter(s) were not significantly different according to Duncan's Multiple at Range test 5% level.

crop and first ratoon) was investigated over 6 years (1988-1994), and the results indicated that the application of 138 g N/mat was the best rate for growth and yield. Bulter (1960) reported that substantial increase in mean weight of bunch and total production per hectare were mainly obtained by application of nitrogen-containing compounds especially when those applied in small quantities. Fruit green life is a very important parameter in the banana industry. Hence, fruit green life and TSS content are used as criteria for screening and selection of promising banana clones for commercial production (Dadzie and Orchard, 1997). Application of fertilizers combinations influenced the reproductive characteristics of Grand Nain. The results as indicated in Tables 2 and 3 showed that there were highly significant increase in growth resulted from all fertilizer treatments reflected in earlier shooting and shorter time to flowering and harvest

in Dwarf Cavendish compared to Grand Nain clones in the two seasons, the time to harvest was 198 days in dwarf and 142 days in Grand Nain. Robinson (1995) stated that the duration from flowering to harvesting of Cavendish subgroup range between 110 and 240 days in subtropical areas. Also the data depicted in Table 2, compost and manure plus urea, and urea alone, resulted in earlier shooting and less time from shooting to harvesting. This result is in agreement with Arunachalam et al. (1976) who reported that excessive nitrogen levels increased the length of maturation period while adequate nitrogen levels caused earlier inflorescence and shortened the time from planting to shooting by 16 days and decreased maturation period by 14 days and thus the whole cycle by one month.

The results presented in Table 4 showed that total soluble solids (TSS) in green mature fruit were significantly higher

in plants treated with fertilizer, and highly significant in the two cultivars in the two seasons, so the effect of the treatment of compost+Urea on TSS in green fruit and TSS in mature fruit in Grand Nain cultivar is more than in Dwarf Cavendish, this was in agreement with Dadzie and Orchard (1997) who compared the total soluble solids of the cultivars Grand Nain and Williams with those of the genotypes FHIA-01 and FHIA-02. They found that the mean total soluble solids content of Grand Nain and Williams were comparable but significantly higher than those of FHIA-01 and FHIA-02 (Dadzie and Orchard, 1997). Since the amount of total soluble solids (TSS) in fruits usually increases as they ripen, they can be a useful index of stage of ripeness. Dadzie and Orchard (1997) reported that sugars form the main component of total soluble solids in ripe fruit, which resulted from starch degradation.

Conclusion

Application of four levels of NPK (18:18:18) 200 5 g/mat/year, urea 400 5 g/mat/year, and organic fertilizer (compost chicken manure) for the two cultivars of Cavendish group, greatly influenced yield characteristics including total bunch weight, total fruit weight, hand weight and number of fingers and fruits per hand. However, fertilizers had significant effects on TSS of the fruit. The highest values of yield components were significantly greater in both, plant crop and first ratoon crop, in weight of bunch, hand and fingers and in the average number of hands and fingers per bunch and fingers per hand. In addition to that there was significant difference in length of finger in both crops. The organic fertilizer with urea and NPK was the best. There were shorter days from planting to shooting and from shooting to harvesting for the two seasons. Combination of fertilizers gave the highest values than the single fertilizer and the control.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Effects of mycorrhiza, organo-mineral and NPK fertilizer on the performance and post harvest quality of sweetcorn

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A field experiment was carried out to assess the effects of vesicular arbuscular mycorrhiza (VAM), Organo-mineral fertilizer (OMF) and NPK (15:15:15), on the performance and post-harvest quality of sweet corn. The experiment was laid out in a split plot design with mycorrhiza as main plot and fertilizer combinations as treatments. VAM inocula were applied at a rate of 888.88 kg ha⁻¹, OMF at 2000 kg ha⁻¹ and NPK (15:15:15) at 400kg ha⁻¹. Storability of the sweet corn was evaluated at open shelf (25-27°C), normal refrigeration (40°C) and deep freezing conditions (0°C). Results showed that there were significant differences ($p < 0.05$) between mycorrhiza and non mycorrhiza. Across mycorrhiza treatment, the highest yields of free corn ears were obtained with the complementary use of OMF and NPK (15:15:15). Fertilizer application had significant effect on the storage life of sweet corn, however, sweet corn stored by freezing at 0°C had the best storage properties in terms of firmness, weight loss and disease incidence.

Key words: Vesicular arbuscular mycorrhiza; sweetcorn yield; Organo-mineral fertilizer

INTRODUCTION

Sweet corn (*Zea Mays*, L.) is gradually becoming an important vegetable crop in Nigeria, since it forms a useful ingredient in the preparation of salad and other food both at home and in hotels (Akintoye and Olaniyan, 2012). It is one of the few vegetables that originated in North America. Canned sweetcorn in salads is popular in Europe. Sweet corn powder can be use as thickening agents for soup and noodles. It has a lower calorie and higher protein concentration compared to the field corn; it is also a good source of dietary fiber.

In Nigeria, sweetcorn production and utilizations have not been a viable option to farmers despite the numerous benefits and economic importance. This is due to the decline in soil fertility, which prevents optimum yield, and the short shelf life of sweet corn. Akintoye and Olaniyan (2012) noted sweet corn as a heavy feeder requiring high amount of macro nutrients. With management practices such as continuous cropping and reduced fallow periods, tropical soils can hardly support cropping without supplementations (CIMMYT 1989/90).

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Although there are environmental criticisms towards the use of inorganic fertilizer (Stone et al., 1995; Dormaar and Chang, 1995; Akintoye and Olaniyan, 2012), its use is still a must (Hera, 1996), since the land is limited and the demand for higher production is pressing. Despite the global campaign towards organic fertilizers, it must be realized that their cost and other constraints frequently deter farmers from using them in recommended quantities and in balanced proportions. Hence, a judicious combination of mineral fertilizers with organic sources of plant nutrient is being promoted. In the same vein, through biological based technology, crop production can be economical and sustainable with emphases on enhanced production, through increased resource productivity on farms with limited resources in which mycorrhiza comes to play.

Food storage at all levels of production is essential since no producer will increase his production if he has no way of storing the excesses. A huge economic loss to farmers results from post-harvest produce wastages as a result of lack or insufficient storage facility. There has been a consensus by authors regarding deteriorations and post-harvest loss of fruits and vegetables in all countries (Cappellini and Ceponis, 1984; Harvey, 1978), thus, the aim of good storage facility is to reduce this waste to the barest minimum. Brecht (2004) stated that storing sweet corn at 30°C for 24 h reduced the sugar content by 60%. Although there is limited information on the post-harvest quality of sweetcorn in Nigeria, several storage techniques such as perforated package (Riad and Brecht, 2002), shrink-wrapping (Aharoni et al., 1996), modified atmosphere (Morales-Castro et al., 1994) and controlled freezing-point (Shao and Li, 2011) have been critically investigated elsewhere.

Consequently, this study was designed to evaluate the effects of mycorrhiza, organo-mineral fertilizer (OMF) and inorganic fertilizer on the performance and storability of sweetcorn under an open shelf, normal and freezer conditions.

MATERIALS AND METHODS

Pre-cropping soil sampling

Before ploughing, soil sampling was done at the depth of 15 cm and 30 cm which was assumed to be the active rooting zone for corn. Sampling was done based on the history of the land. Composite samples were taken from the plots. These were bulked and air-dried in the shade for 7 days after which the samples were grounded and thoroughly mixed. They were then sieved using 2 mm and 0.5 mm sieves. These prepared samples were analyzed for physical and chemical properties at the analytical laboratory of the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria.

Experimental design

A field experiment was conducted in the National Horticultural

Research Institute (NIHORT), Ibadan Nigeria (7° 54¹ N; 7° 30¹ E), to determine the effects of mycorrhiza, pacesetter OMF (Grade A) and golden fertilizer NPK (15:15:15) on the performance and storability of sweet corn. The vesicular arbuscular mycorrhiza (VAM) was obtained from IITA, Ibadan. The variety of sweet corn used was T21035 (*Zea mays* L.) The corn was sourced from the NIHORT, Ibadan. The experiment was laid out on split plot design with a 2 x 2 x 2 factorial arrangement. Mycorrhiza inoculation formed the main plot treatment, the application of OMF and NPK (15:15:15) were sub-plot treatments with three replicates. Plot size measured 3 m x 2 m (6 m²) and separated by 2 m alleyways between the main plots and 1 m alleyways between the sub-plots. The experimental plot was harrowed twice before planting. Plant spacing of 75 cm x 30 cm was used to give a plant population of 44, 444 plants ha⁻¹. 20 g of VAM fungal inocula were placed few centimeters below the sweet corn seedling root in each VAM fungus treatment, making up an application rate of 888.88 kg ha⁻¹. OMF at the rate of 2000 kg ha⁻¹ and NPK (15:15:15) at the rate of 400 kg ha⁻¹ were applied simultaneously in each fertilizer treatment. Fertilizer application was done in two splits, at 14 and 42 days after planting (DAP). Weeding was done manually at 20 and 35 DAP, subsequent weed emergences were hand pulled to ensure a weed free field.

Growth and yield assessment

Measurements on plant height, stem girth, number of leaves and leaf area were taken weekly. Leaf area was calculated according to the model given by Saxena and Singh (1965) as:

$$0.75 \times \text{Length} \times \text{Width}$$

Harvesting of sweet corn was done at 98 DAP when the cobs were still green.

Storage experiment

Harvested sweetcorn was stored under three storage conditions viz: ambient condition (25-27°C, RH 83%), refrigeration (4°C, RH 87%) and deep freezing (0°C, 90%). Data were collected on storage weight loss.

$$\text{Loss in fresh weight (\%)} = \frac{\text{Difference in weight}}{\text{Initial weight}} \times 100$$

Firmness and decay or mould growth were subjectively scored. Firmness was determined by hand-feel and rated on a scale, where 1= firm, 2=moderately firm, 3=moderately soft and 4= soft. Mould growth on cobs with grains were observed and rated as 1= intact, 2= slight attack, 3= high incidence, 4=unmarketable.

Data analysis

Analysis of variance procedures were performed for all data to test treatments effects on the various parameters measured. Significant means ($p < 0.05$) were separated using Duncan's Multiple Range test (Duncan, 1955). Data were analyzed using Statistical Analysis System software, version 8.0 (SAS, 1999).

RESULTS AND DISCUSSION

Field experiment

The soil was found to be sandy loamy on textural triangle and slightly acidic (pH 6.0) (Table 1). Total N value of

Table 1. Pre-planting soil physico-chemical properties

Properties	Soil
pH (H ₂ O)	6.60
Total N (%)	1.08
Bray-1- P	4.93
Organic C (%)	1.80
Exchangeable cation (cmol/kg)	
K	0.11
Mg	1.50
Ca	2.00
Na	0.43
Exchangeable acidity (cmol kg⁻¹)	
CEC	0.60
Mn	27.69
Fe	9.66
Cu	0.80
Zn	2.80
Particle size analysis (g kg⁻¹)	
Sand	810
Silt	120
Clay	70

Table 2. Effects of mycorrhiza on the number of leaves, plant height, leaf area and stem girth of sweetcorn.

Parameter	Mycorrhiza effect	2 WAP	4 WAP	6 WAP	8 WAP
Number of leaves	Mycorrhiza	5.25 ^a	5.92 ^a	8.75 ^a	10.75 ^a
	Non-mycorrhiza	4.92 ^a	5.08 ^a	6.83 ^a	7.50 ^b
Plant height (cm)	Mycorrhiza	10.33 ^a	43.08 ^a	136.55 ^a	167.00 ^a
	Non-mycorrhiza	9.93 ^a	36.50 ^b	109.74 ^a	133.11 ^b
Leaf area (cm ²)	Mycorrhiza	36.43 ^a	207.10 ^a	422.68 ^a	497.24 ^a
	Non-mycorrhiza	32.13 ^a	171.59 ^b	306.78 ^b	409.76 ^b
Stem girth (cm)	Mycorrhiza	2.24 ^a	3.75 ^a	5.50 ^a	7.65 ^a
	Non-mycorrhiza	1.78 ^b	3.23 ^b	4.92 ^a	5.93 ^b

^{ab}Means followed by the different superscripts along same column are significantly different at 5% level of probability.

1.08%, was less than the critical level of 1.15% (Enwezor et al., 1989) and the phosphorus level was found to be lower than 10-16 mg kg⁻¹ critical level reported by Adeoye and Agboola (1985). The 0.11 cmol kg⁻¹ obtained in this study for potassium was less than the critical level of 0.18-0.20 cmol kg⁻¹ (Agboola and Obigbesan, 1975). This result suggests that the soil used for the study was very low in major nutrient elements and would respond favourably to fertilizer application.

The effect of mycorrhiza on the growth parameter is presented in Tables 2 and 3. At 2 WAP, there was significant effect of mycorrhiza inoculation only on stem girth ($p < 0.05$). Leaf area and stem girth were significantly different between the inoculated and non-inoculated plants at 4, 6 and 8 WAP with mycorrhiza inoculated sweetcorn having the highest significant means throughout. Although mycorrhiza inoculation significantly affected all growth parameters, the time of expression varies. As the

Table 3. Interactive effects of mycorrhiza and fertilizer on stem girth of sweetcorn.

Mycorrhiza	Fertilizer	2 WAP	4 WAP	6 WAP	8 WAP
Mycorrhiza	NPK	2.18 ^b	3.93 ^a	5.47 ^{ab}	7.67 ^{ab}
	OMF	2.08 ^b	3.67 ^b	5.20 ^{bc}	7.87 ^a
	NPK+OMF	2.72 ^a	4.17 ^a	5.47 ^{ab}	7.67 ^{ab}
	Control	1.96 ^{bc}	3.23 ^c	5.87 ^a	7.40 ^b
Non-mycorrhiza	NPK	1.72 ^{cd}	3.42 ^{bc}	4.90 ^{bc}	6.00 ^{cd}
	OMF	1.76 ^{cd}	3.20 ^c	4.93 ^{bc}	5.80 ^{cd}
	NPK+OMF	2.09 ^b	3.33 ^c	5.13 ^{bc}	6.20 ^c
	Control	1.55 ^d	2.93 ^d	4.70 ^d	5.53 ^d

^{abcd}Means followed by the different superscripts along same column are significantly different at 5% level of probability.

Table 4. Interactive effects of mycorrhiza and fertilizer on yield of sweetcorn.

Mycorrhiza	Fertilizer	Yield (kg ha ⁻¹)
Mycorrhiza	NPK	3339.20 ^b
	OMF	2858.20 ^c
	NPK+OMF	3514.96 ^a
	Control	2781.37 ^c
Non-mycorrhiza	NPK	1842.27 ^e
	OMF	1144.43 ^f
	NPK+OMF	2418.63 ^d
	Control	971.43 ^g

^{abcdefg}Means followed by the same letter in each column are not significant different by Duncan's multiple range test at 5% level of probability.

mycorrhiza effects manifestation started as early as 2 WAP on the stem girth, number of leaves did not increase significantly until 8WAP. The reason for this variation is not far from the difference in the physiological requirements of different plant parts. The reliability of VAM to enhance the P uptake of the crop has been reported to vary with length of fallow, tillage practices, soil nutrient status, fertilizer and host crop (Thompson, 1994). This was confirmed in the present study as leaf area showed appreciable increase with mycorrhiza and fertilizer treatment combinations over the plant without mycorrhiza and fertilizer. The interactive effect of mycorrhiza and fertilizer combinations on the yield of sweet corn is presented in Table 4.

Irrespective of the type of fertilizer used, sweet corn with mycorrhiza generally had more yield than their counterparts with no mycorrhiza. The significant increase in sweet corn yield for mycorrhiza-inoculated plants underscores the positive effects of VAM in plant nutrition. The reliant of plant nutrition, especially phosphorus uptake, on VAM have been documented (Howler et al., 1987; Osonubi et al., 1991; Atayese et al., 1993; Fagbola et al., 1998). Tas (2014) also reported a higher yield for

sweet corn inoculated with mycorrhiza. With mycorrhiza inoculation, combination of NPK with OMF gave the highest significant yield. This result is in variance with the report of Akintoye and Olaniyan (2012) who obtained higher yield from plot with NPK relative to organic fertilizer and organo-mineral fertilizer, similar report was also documented by Paul and Beauchamp (1993). However, the better yield under the complementary use of OMF and NPK has been reported by several authors (Titiloye, 1982; Akpomudjere and Omueti, 1991; Sodunke, 1997).

Storage experiment

The three storage methods showed significant differences ($P < 0.05$) for the three post harvest indices (Table 5). While storage in deep freezer was best across the three indices used, storage under ambient condition was the worst. The result showed that loss in quality of sweet corn was greatly slowed down by storage under cold freezer conditions. Better storability of sweet corn in deep freezer than in the normal refrigerator and open

Table 5. Effect of storages methods on percentage disease incidence, percentage weight loss, and firmness of sweetcorn.

Storage method	Disease	Weight loss (%)	Firmness (1-4)
Ambient	3.50 ^a	35.46 ^a	2.2. 2.66 ^a
Normal	2.00 ^b	21.78 ^b	2.45 ^a
Deep freezing	1.10 ^c	2.54 ^c	1.10 ^b

^{abc}Means followed by the same letter in each column are not significant different by Duncan's multiple range test at 5% level of probability.

shelf as indicated by firmness, percentage weight loss and percentage disease incidence agrees with the report of Willis et al. (1981). The authors reported that temperature and humidity were the most important factors in the shelf life of fruit and vegetables. Also, the observed reduction in spoilage of sweetcorn due to refrigeration was in line with the report of Babatola and Olaniyi (1998).

Conclusions

Mycorrhiza inoculation and the complementary use of organo-mineral and inorganic fertilizer are very essential for enhancing soil nutrient status and increasing crop yield. Storage of sweetcorn is very important for prospects in its production, as corn stored in deep freezer was better than those in the normal refrigerator and open shelf in terms of firmness, percentage weight loss and disease incidence.

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Full Length Research Paper

The use of electrolyte leakage procedure in assessing heat and salt tolerance of Ruzaiz date palm (*Phoenix dactylifera* L.) cultivar regenerated by tissue culture and offshoots and treatments to alleviate the stressful injury

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Salt and heat stresses are one of the greatest constraints facing agricultural production worldwide, particularly in arid and semi-arid countries where scarcity of water and high temperatures prevail. Assessing tolerance level of date palm trees regenerated by tissue culture against heat and salt stresses is a prerequisite. This investigation aims to determine heat and NaCl tolerance of date palm (cv. Ruzaiz) produced by tissue culture and offshoots as well as to study the possibility of increasing tolerance to heat stress alone or in the presence of NaCl stress by using calcium, potassium or oleic acid by using electrolyte leakage method which based on sigmoidal curves at 50%. Tissue culture plants used in this investigation were at 4 and 10 months old vitro plants namely; VP2 and VP3, respectively as well as offshoots from the same variety attached to mother plant in the field. Electrolyte leakage method was used to determine thermo-tolerance of leaf tissues. Leaf segments of five-centimeter length from VP2, VP3 and offshoots were assayed in the laboratory for tolerance to treatments (heat, heat plus NaCl, heat plus KCl, heat plus CaCl₂, heat plus oleic acid, heat plus NaCl and oleic acid, heat plus NaCl and KCl and heat plus NaCl and CaCl₂). The concentrations of the test compounds were: NaCl at 1% w/v, KCl or CaCl₂ (0.2 M) or oleic acid (0.1 M). A completely randomized design was used with three replications. The results revealed that thermo-tolerance values were 53, 53.5, and 58.5°C for VP2, VP3, and offshoot leaflets, respectively. Also there is a potential to increase the thermo-tolerance of VP2, VP3, or offshoots that could increase their survival under field conditions by pretreatment with KCl, CaCl₂ (0.2 M) or oleic acid (100 ppm).

Key words: Electrolyte leakage, thermo-tolerance, membrane stability, salinity, lethal temperature, heat regime, semipermeability.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a dioecious long-lived monocotyledon tree. It is a major crop in the Arabian Peninsula (Youssef and Wad, 2008). Offshoots are a

dominant multiplication method for date palm trees. However, it does not meet high demand due to a limited number of offshoots produced from each tree. Plant

tissue culture technique has been used on large scale to reproduce homogenous date palm plants that are true to type and free from diseases (Zaidabd de Wet, 1999; Awad et al., 2006). However, there is lack of information about the tolerance of regenerated *in vitro* plants to heat and salt stresses. These stresses are usually associated with slow growth rate and frequent death of plants after transplanting (Awad et al., 2006; Hammouda et al., 1998).

The agronomic importance of date palm is linked to its high tolerance to environmental stresses, such as salinity, drought and high temperature for limited levels as compared to many other fruit trees (FAO, 1982; Ramoliya and Pandey, 2003). To our best knowledge, till now there is no study so far that identified the exact tolerance level or the lethal temperature of heat and salt stresses to date palm offshoots or *in vitro* plants for Ruzaiz cultivar. Electrolyte leakage procedure has been used effectively to measure cell membrane stability due to environmental stresses such as water, drought, heat and cold stresses in date palm crop (Youssef and Wad, 2008; Awadet al., 2006; Farag and El-Konaissi, 2002) and other crops (KalimUllah et al., 2014; Adil et al., 2014; Anbu and Sivasankaramoorthy, 2014; Cha-um et al., 2013; Asemoto and Conaire, 2010; Cha-Um et al., 2010). Youssef and Wad (2008) found that the electrolyte leakage of date palm seedlings positively correlated with increasing salinity and they considered that as indications of the degree of impairment to membrane integrity.

In nature the plant is exposed to multiple stresses which interact to limit growth and productivity (Turner and Kramer, 1980). Al-Mulla et al. (2013) found that Khalas date palm cultivar tolerates water salinity to 20 dS/m and 'Nabusaif' was the least tolerant cultivar. Similarly, Al-Abdoulhad 2011 found Saudi Arabia's premier date cultivar (Khalas) was the most salt tolerant cultivar. Alrasbi et al. (2010) concluded from their experiment that date palm seedlings of varieties 'Khalas', 'Khunaizy' and 'can be irrigated with saline water during vegetative growth till 9 dS m⁻¹, however water with E_c 18 dSm⁻¹ caused a significant decline in growth will reach up to 50%

There are many reports of a protective effect of Ca²⁺, K⁺ and unsaturated fatty acids such as oleic acid against heat or salt injury. Gary-boo (1970) reported that calcium might serve to bind the polar head groups of phospholipids together and thus limit membrane permeability. This was reflected on reduced electrolyte leakage. Poovaiah and Leopold (1976) also reported that the leakage of solutes from plant tissues induced by (NH₄)₂SO₄ could be relieved by the addition of CaCl₂. Similarly, Toprover and Glinka (1976) found that Ca²⁺ inhibited the reversible; heat-induced efflux of betacyanin

from beetroot exposed to 45°C for 90 min. Richard and Gray (1984) reported that CaCl₂ could protect leaf membranes against the leakiness induced by NaCl. Similarly, Ben-Hayyim et al. (1987) found that K⁺ application could reduce the deleterious effect of salinity on plant development. Darwesh (2013) found that application of amino acids had significantly ameliorated the harmful effects of salinity on date palm plantlets cv. Bartomouda. However, efficiency of Ca²⁺, K⁺ and oleic acid in alleviating or increasing heat or salt injury for Ruzaiz date palm has not studied yet.

The objectives of this study were to accurately identify the heat and salt tolerance of date palm plants (Ruzaiz) cultivar reproduced by tissue culture and offshoot as well as to study the possibility of increasing tolerance to heat stress alone or in the presence of sodium chloride stress by calcium and potassium, as well oleic acid.

MATERIALS AND METHODS

This study was conducted in the Horticulture laboratory at the Department of Plant Production, Faculty of Food Systems, United Arab Emirates University in 2002. Six and ten months old date palm *in vitro* plants (VP2 and VP3, respectively) regenerated by organogenesis tissue culture technique and 4 years old offshoots attached with mother plant of Ruzaiz date palm cultivar were used in this study. For more hardening, selected tissue culture plants were maintained for three weeks in controlled greenhouse (23 ± 1°C, 40-50% relative humidity) at Date Palm Research and Development Unit at Al-Oha Research Station. Offshoots leaves were detached and directly used. Electrolyte leakage procedure was used to measure cell membrane thermo-stability after exposure to a heat regime. This regime followed the treatment with NaCl (1% w/v) or CaCl₂, KCl, or oleic acid. To determine the thermo-tolerance of plants, uniformed thirty-three fully expanded leaves of 30 cm length (3 leaves/plant) were collected. Leaves were washed with tap water and rinsed in deionized water to remove the dusts and electrolytes adhering to the surfaces and lightly cleaned with tissue papers. Leaf segments of five-centimeter length were cut from the middle of each leaflet and placed in each test tube. In each tube, 1 ml of deionized water was placed to prevent tissue desiccation and loosely covered with aluminum foil. All test tubes were placed in a water bath shaker (Thermostat) and tissues were exposed to a heat regime ranging from 30-75°C at 5°C increments for 30 min. Three tubes per plant stage remained at 22°C as controls. At the end of the 30 min exposure, leaflet segments were removed from the water bath, cut into about 1 mm strips to allow uniform diffusion of electrolytes, returned to the tubes along with 40 ml of deionized water, and incubated in refrigerator at 7°C overnight to allow more diffusion of electrolytes from treated damaged tissues before electrical conductivity of each solution was determined. In the next day leaf segments were taken out from refrigerator, warmed up to room temperature (22 ± 2°C) and placed in a shaker for 1 h to diffuse electrolytes, then electrolyte leakage, before killing, was measured with electrical conductivity meter. Leaf segments were then killed by autoclaving (121°C) for 10 min, after that were left on the shaker for 1 h to diffuse electrolytes, then the total electrolyte leakage reading were taken by using the same conductivity meter.

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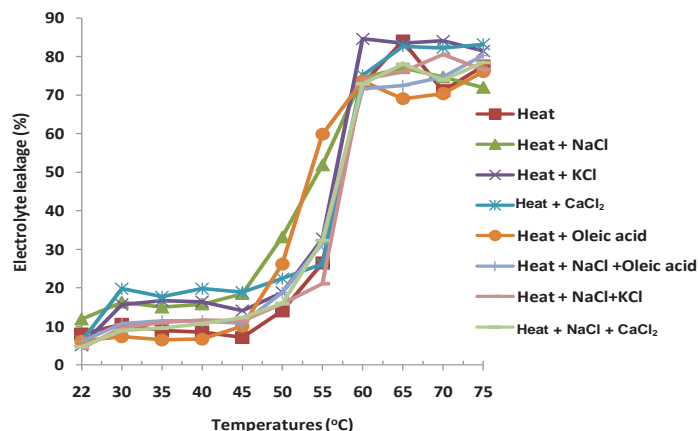


Figure 1. Lethal temperatures for VP2 plants of Ruzaiz date palm cultivar as influenced by various treatments.

Percentage of electrolyte leakage before killing to after killing was calculated according to Farag and El-Konaissi (2002) and Awad et al. (2006). The three replications of the control tissue went through the same procedure to determine percentage of electrolyte leakage. The experiment consists of 8 treatments, each treatment replicated 3 times, and each leaflet represents one replication. A completely randomized design was used.

To assess the thermo-tolerance of leaflets in the presence of high salt concentration, the same procedures as in heat test were followed, except leaflet segments of 5-centimeter length were cut into two pieces to enhance penetration of salts and immersed in 1% (10,000 ppm) of NaCl solution for hour. Segments were plotted dry with tissue papers then placed in test tubes with 1 ml of deionized water in each tube to prevent tissue desiccation. With regard to assessment of thermotolerance after certain treatments, leaf segments were taken and dipped for 1 h in either KCl, or CaCl₂ (0.2 M), or Oleic acid (0.1 M). Furthermore, 100 ppm of oleic acid was dissolved in 50 ml of ethanol. These segments were removed from the solutions and gently plotted dry with tissue paper. One segment was then placed in a test tube that already contained 1 ml of deionized water. Tubes of various treatments were exposed to the heat regime (from 30 - 75°C with 5° increments). At each temperature, three leaf segments of each treatment were removed from the water bath shaker. Measurements of electrolyte leakage and calculations of the percentage of this leakage before and after killing were determined according to Farag and El-Konaissi (2002). Three replications were used with each treatment in a completely randomized design. All data were analyzed using MSTATC program and the least significant difference (LSD) at 0.05 level was used to compare the means.

RESULTS

Generating the sigmoidal curves

As shown in Figures 1 to 3 the shape of the sigmoidal curve was consistent in all treatments. The trend of results was so clear that there was no need to fit a curve but connected the actual points. The lethal temperature was determined at 50% electrolyte leakage. Figures 1 to 3 for VP2, VP3 and offshoots plantlets, respectively represent the results of heat tolerance alone or after

treatments with sodium chloride, potassium chloride, calcium chloride, oleic acid, or in other combinations. The sigmoidal curve started with slow increase in electrolyte leakage then at a temperature that varies from one treatment to another it increases varies rapidly. After exceeding 50% electrolyte leakage, there is a leveling off with very minor changes in this leakage with increase in the temperature. Thus, the figures clearly show inflection of the line.

Identification of leaflets thermotolerance

VP2 plants

Differences in lethal temperatures after different treatments of VP2 plants are presented in Figure 1. From the inflection point at 50% electrolyte leakage, it was evident that the lethal temperature for VP2 was at 53°C. Furthermore, when these leaflet segments of plantlets were immersed in NaCl (1 %w/v) for hour before the exposure to the heat regime, the lethal temperature was 53.5°C. At this stage of plant age, tissues were not sensitive to used salt concentration However, when the tissues were treated with either potassium chloride or calcium chloride, heat tolerance of VP2 stage was increased as indicated by the lethal temperatures (57 and 57.5°C, respectively) (Figure 1), as compared with heat treatment alone. Thus, increasing tissues content of calcium or potassium could have a direct effect on increasing heat tolerance of date palm plantlets at this stage of acclimatization. Heat tolerance of oleic acid-treated tissues was slightly increased (the lethal temperature was 53.4°C). However, a marked change of lethal temperature for VP2 stage was obtained when the leaflet segments were pretreated with the salt plus oleic acid or KCL and CaCl₂ before the exposure to the heat regimes. The lethal temperature for heat plus salt and oleic acid was 57.1°C, while for heat plus salt and KCl and CaCl₂ were 58 and 57.1°C, respectively.

VP3 plants

Figure 2 shows the lethal temperature of leaflet segments of VP3 plantlets whether directly exposed to the heat regime or after treatments with NaCl, KCl, CaCl₂ or oleic acid. The heat tolerance of tissue did not significantly vary from those tissues exposed to heat in the presence of NaCl (1% w/v) (Figure 2). Each of KCl and CaCl₂ treatments increased the thermotolerance of leaflets tissues at this stage than non- treated. The lethal temperatures for heat plus KCl or plus CaCl₂ treatments were 56.5 and 57°C, respectively. A direct positive effect on the thermotolerance was also obtained when VP3 leaflets tissues were pretreated with oleic acid (100 ppm) before the exposure to the heat regime. The lethal

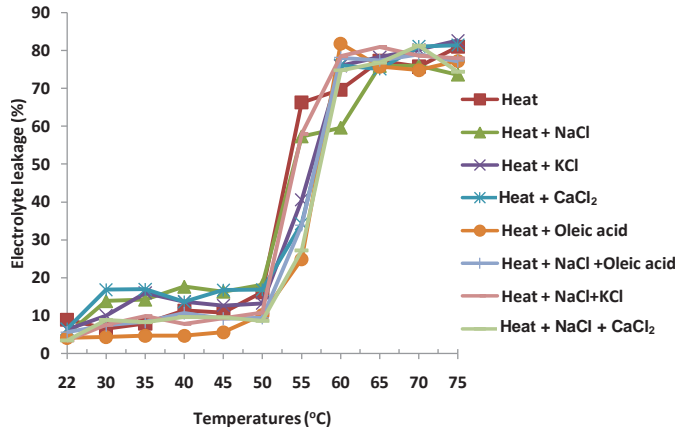


Figure 2. Lethal temperatures for VP3 plants of Ruzaiz date palm cultivar as influenced by various treatments.

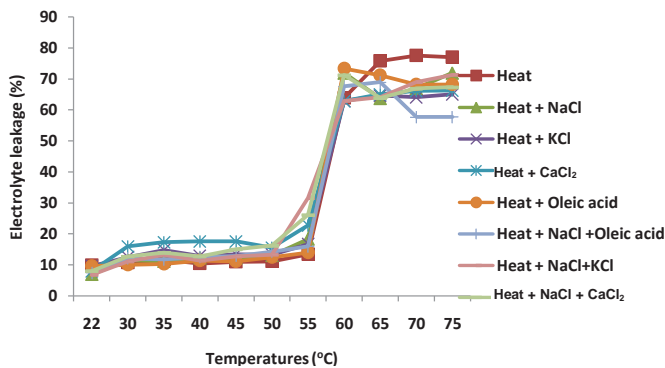


Figure 3. Lethal temperatures for offshoots plants of Ruzaiz date palm cultivar as influenced by various treatments.

temperature of heat plus oleic acid was 57.1°C. At this stage the response of the tissue to presence of oleic acid was greater than that obtained with VP2 stage. The lethal temperature of heat plus NaCl and oleic acid (Figure 2) was 57°C and not differ from that obtained with heat plus oleic acid since this salt concentration had no adverse effect on the thermo-tolerance of tissues. Furthermore, when the segments were pretreated with NaCl and KCl, their lethal temperature was 54°C. Calcium treated tissues even in the presence of salt maintained their improved thermo-tolerance where the lethal temperature was 57.4°C.

Offshoots

Lethal heat temperature of leaflets taken from Ruzaiz offshoots (58.5°C) presented in Figure 3 was much higher than that of VP2 and VP3 leaflets (Figures 1 and 2). These offshoots were still attached to mother plant under harsh conditions in the field. The offshoots went through a hardening process that was reflected on their

thermo-tolerance. In a similar way NaCl at the used concentration did not have an adverse effect on the leaflets (Pinnae) thermo-tolerance, as it was 58°C (Figure 3). There was no added advantage on the heat tolerance of offshoots leaflets when they pretreated with KCl or CaCl₂. Even oleic acid did not improve the tolerance to heat stress which indicates again that 58.5°C could be the maximum potential of these tissues to tolerate heat stress.

The interaction between the heat regime and VP2, VP3, and offshoots

The data in Table 1 indicated that leakage of electrolytes was significantly higher at VP2 stage than that obtained with VP3 stage even at relatively low temperatures such as 30, 35, 40, 45 and 50°C. However, at 55°C, both VP2 and VP3 leaflets had similar electrolyte leakage. Similar results were obtained at the high temperatures 60, 65, 70 and 75°C. These results are in agreement with that found in Figure 1 and 2 where the lethal temperatures for VP2, and VP3 stage were 53 and 53.5°C, respectively. Although these lethal temperatures were not very different from each other but the injury to VP2 leaflets started earlier than that occurred to VP3 even though it was not lethal prior to 50°C. If electrolyte leakage of VP2 leaflets was compared with that of offshoots, the data in Table 1 showed a similar trend to that found above between VP2 and VP3. Again, electrolyte leakage of VP2 leaflet segments was significantly higher than that of offshoots at the temperatures 30, 35, and 40°C and even at the sublethal temperature 50°C. Although leakage of electrolytes of VP2 at 55°C reached to the lethal value for VP2 tissues (64.1%), it was found that, at this temperature, electrolyte leakage was only 13.4% for offshoot leaflets. Even after exceeding the lethal temperature, electrolyte leakage of VP2 was still significantly higher than that obtained with offshoots at 75°C. When electrolyte leakage of the relatively more advanced stage in acclimatization (VP3) was compared with the field-hardened offshoots, we found that this leakage was not statistically different (P<0.05) at 30, 35, 40, 45 and 50°C. However, at 55°C, electrolyte leakage of VP3 leaflets was significantly higher than that of offshoots (66.2 and 13.4%, respectively). This was supported by the finding in Figure 3 and 2 where the lethal temperature for offshoot leaflets was 58.5°C and for VP3 leaflet was 53.0°C. After exceeding the lethal temperatures for both VP3 and offshoots, electrolyte leakage was very high (over 70 %) and similar for both. The data in Table 1 also indicates that as the VP2 leaflets lost 18.3% electrolytes at 35°C, while the offshoot leaflets lost only 13.4% at 55°C. Although this difference was not statistically significant (P<0.05), but the leakage was achieved at much higher temperature with the offshoot leaflets. This proves the importance of the duration factor in heat stress of date palm plantlets that could be

Table 1. The effect of interaction between the heat regime and the stages of acclimatization and hardening of Ruzaiz date palm plants on electrolyte leakage (%).

Temperature regimes (°C)	VP2*%	VP3*%	Offshoot*%	Mean
Control (22)	11.4 **ijkl	8.9 ^{kl}	10 ^{kl}	10.1
30	18.5 ^{gh}	6.5 ^l	10.7 ^{ijkl}	11.9
35	18.3 ^{gh}	7.9 ^{kl}	11.6 ^{ijkl}	12.6
40	19.4 ^g	11.3 ^{ijkl}	10.5 ^{ijkl}	13.7
45	16.6 ^{ghi}	10.6 ^{ijkl}	10.9 ^{ijkl}	12.7
50	36.3 ^f	16.1 ^{ghij}	11.3 ^{ijkl}	21.2
55	64.1 ^e	66.2 ^e	13.4 ^{hijk}	47.9
60	73.2 ^{cd}	69.5 ^{de}	63.8 ^e	68.8
65	76.4 ^{abc}	77.2 ^{abc}	76.3 ^{abc}	76.6
70	75.0 ^{bcd}	75.7 ^{bc}	77.5 ^{abc}	76.1
75	81.8 ^a	80.9 ^{ab}	74.3 ^{cd}	79.0
Mean	44.6	39.2	33.7	39.2

L.S.D. (0.05) = 5.925; C.V. % = 9.27. ^{*}Values are means of three replications (each replication represents date palm plantlet or offshoot). **means within columns or rows followed by same letter do not differ significantly at 0.05 level (least significant difference test).

Table 2. The effect of interaction between treatments and the stages of acclimatization and hardening of Ruzaiz date palm plants on electrolyte leakage (%).

Treatments	VP2 *%	VP3*%	Offshoot *%	Mean
Heat	44.6 **a	39.2 ^{bcd}	33.7 ^{ghij}	39.2
Heat and NaCl	42.2 ^{ab}	38.9 ^{bcd}	32.7 ^{hij}	37.9
Heat and KCl	41.1 ^{abc}	39.0 ^{bcd}	32.2 ^{ij}	37.4
Heat and CaCl ₂	42.2 ^{ab}	39.3 ^{bcd}	34.5 ^{fghij}	38.7
Heat and oleic acid	37.5 ^{cdefg}	33.4 ^{ghij}	32.7 ^{hij}	34.5
Heat and salt and oleic acid	36.2 ^{defghi}	36.0 ^{defghi}	30.7 ^j	34.3
Heat and salt and KCl	35.7 ^{defghi}	38.4 ^{bcdef}	33.3 ^{hij}	35.8
Heat and salt and CaCl ₂	36.4 ^{defgh}	34.8 ^{efghij}	34.0 ^{ghij}	35.1
Mean	39.5	37.4	33.0	36.6

L.S.D. (0.05) = 4.14; C.V. % = 7.05; ^{*}values are means of three replications (each replication represents date palm plantlet or offshoot); **means within columns or rows followed by same letter do not differ significantly at 0.05 level (least significant difference test).

addressed in the further studies.

The interaction between treatments and VP2, VP3, and offshoots

Differences in electrolyte leakage of the two acclimatization stages VP2, VP3 and the field-hardened offshoots in relative to heat treatments are shown in Table 2. The interaction between various treatments and the two acclimatization stages (VP2 and VP3) did not show a significant difference in electrolyte leakage between treatments except with the heat treatment alone for VP2 and VP3. The data also indicated that VP2 leaflet lost significantly more electrolytes than VP3 leaflet (Table 2). When the leakage of VP2 leaflets was compared with that obtained with offshoot leaflets, it was

found that this leakage was higher in VP2 leaflets than that of the offshoot leaflets whether with heat alone or when pretreated with salt, potassium, calcium, oleic acid, or salt plus oleic acid. Similarly, VP3 leaflets had higher electrolyte leakage than offshoot leaflets with all treatments except with heat plus oleic acid treatment and heat plus salt and calcium (Table 2).

DISCUSSION

The sigmoidal pattern of electrolyte leakage obtained in this study, for heat stress alone or for pretreated tissues with NaCl, KCl, CaCl₂, or oleic acid then exposed to heat stress agrees with Farag and El-Konaissi (2002) on Barhi and Khalas date palm cultivar and other studies (Nilsen

and Orcutt, 1996; Agarie et al., 1995; Cha-Um et al., 2010; Anbu and Sivasankaramoorthy, 2014). Similar pattern was also obtained when the leaflet segments were exposed to NaCl plus each of oleic acid, or KCl, or CaCl_2 , and then experienced heat stress. Similarly, Anbu and Sivasankaramoorthy (2014) found that supplementary of calcium chloride (10 and 15 mM CaCl_2) ameliorated electrolyte leakage of rice leaves as a result of salt stress.

Electrolyte leakage is an indicator to the injury occurred to plasma membrane after exposure to stresses. It has been recognized as a valid, reproducible, simple, and quantitative test for assessing cell viability after heat, salt water, or even cold stresses (Adil et al., 2014; KalimUllah et al., 2014; Cha-Um et al., 2010).

Under heat stress, proteins of the plasma membrane denature or aggregate according to the severity of stress and/or membrane lipids becomes hyperfluid. These changes result in increased leakage of electrolytes from the membrane (Levitt, 1980). The current study provided experimental evidences for the differences in heat tolerance between *in vitro* date palm plants and the offshoots grown along with the mother plant under natural conditions. It has been known for long time that date palm plants are tolerant to heat stress (Hammouda et al., 1998). However, no accurate test has been reported to show the exact tolerance level especially for those plantlets reproduced by tissue culture. As shown in results, the thermotolerance was 53.0, 53.5 and 58.5°C for VP2, VP3, and offshoots leaflets, respectively. This information has very important implications since many tissue culture plants could be die if air temperature during the day reached 50°C. Furthermore, we must take in consideration the heat absorption factor where in hot climate heat absorption is higher than heat dissipation. As a result of this, tissue temperature is usually higher than air temperature by at least 10 to 12°C (Levit, 1988). These results should guide date palm growers to the suitable time of the year before transplanting date palm plantlets especially those produced by tissue culture.

Differences between *in vitro* plants and offshoots of date palm are expected since tissue culture plants are produced under delicate microenvironment, while offshoots went through sufficient hardening conditions in the field. The other factor that must be considered that VP2 and VP3 plants can not reflect as much heat as that reflected by offshoots because the tissue culture plants do not have a thick cuticle or similar epicuticular waxes as that found on offshoot leaflets. Thus, the avoidance mechanism of tissue culture plants after these two stages of plants age is less efficient than the hardened offshoots.

With regard to heat stress tolerance in the presence of high salt concentration, the used NaCl in this study did not have an adverse effect on the thermotolerance of VP2, VP3 or offshoot leaflets. This could be due to the exposure to heat stress after salt stress, so there might not be a direct effect of the pretreatment with salt. In

other words, NaCl treatment might need more time to exhibit an injury by heat after that. The other possibility is that following the pretreatment with NaCl, the tissue might need more duration of heat stress. The differences in result were attributed to the amount taken by the tissues. If the tissues permitted more penetration or diffusion of salts, the thermotolerance will be lowered. Like this result was noticed by Husein et al. (1993) who found that growth of date palm seedlings was unaffected by low salinities. Borochoy et al. (1991) finding revealed that an excess of NaCl caused an increase in electrolyte leakage. Similarly, Youssef and Wad (2008) considered that the high electrolyte leakage from of date palm seedlings exposed to high salinity a strong indication of the degree of impairment to membrane integrity. The increase in thermotolerance of calcium- treated tissues, in this study, of VP2 and VP3 leaflets is supported by the findings many other researchers (Gary, 1970; Poovaiah and Leopold, 1987; Leopold et al., 1984; Richard and Gary, 1984). Calcium was reported to play a very vital role in maintaining the plasma membrane integrity. Thus, calcium could protect leaf membrane against leakiness induced by heat stress. The polar head groups of the membrane phospholipids are bound together by calcium that limits membrane permeability and reduce electrolyte leakage (Gary, 1970).

Similar role for potassium was reported. Ben-hayyinum et al. (1987) found that potassium application could reduce the deleterious effect of salinity on plant development. Potassium can also bind to the plasma membrane and maintain its integrity that results in reducing electrolyte leakage. Results shown in Figures 2 and 3 agree with this explanation. There was no added advantage on the thermotolerance of offshoots if the leaflets were pretreated with potassium, calcium, or oleic acid when compared with heat treatment alone. Since the thermo-tolerance of offshoot leaflets was high (58.5°C), this might be the maximum tolerance for such hardened tissues in the field. Hassan and El Samnoudi (1998) found amount of potassium uptakes by date palm seedlings increased with increasing salinity within a moderate range.

Regarding the role of unsaturated fatty acid in increasing the thermotolerance of plants, it was reported by Harwood et al. (1994) that these acids protect the membrane from hyperfluidity that means keeping its integrity. Similarly, Nilsen and Orcutt (1996) reported that membrane thermo-stability at high temperature could be modified by changes in fatty acid unsaturation, the position of fatty acids on the glycerol backbone, the composition of fatty acids, and the abundance and compositional of sterols. Thus, the change in membrane fluidity affects the properties of embedded proteins (enzymes) that make the membrane leakier to electrolytes. In this study, the pretreatment with oleic acid caused an increase in the thermo-tolerance of VP2 and VP3 plants (Figures 1 and 2). These results agree

with what was reported on the positive effects of unsaturated fatty acids on the plasma membrane (Harwood et al., 1994; Nilson and Orcutt, 1996). Effect of the duration factor has been reported in stress studies (Levitt, 1980; Nilson and Orcutt, 1996). Results of this study also propose that. Although the thermo-tolerance between VP2 and VP3 leaflets did not vary much, but electrolyte leakage of VP2 leaflets was significantly higher than that of VP3 leaflets at sublethal temperatures (30-50°C). These results suggested the importance of studying the thermotolerance of this stage at various durations. In general, there is a great potential to increase the thermotolerance of the tissue culture regenerated plants and offshoots by pretreatment them with safe chemicals such as potassium chloride, calcium chloride, or oleic acid.

CONCLUSIONS AND RECOMMENDATIONS

This study showed for the first time a quantitative determination of the thermo-tolerance for tissue culture regenerated plants (VP2 and VP3) and for hardened offshoot of the same Ruzaiz cultivar. The results of heat stress regime alone or pretreatment of leaflets with NaCl, KCl, CaCl₂, or oleic acid then the heat regime resulted in consistently generating a sigmoidal curve as shown in other studies. With regard to multiple stresses heat tolerance of leaflets tissues after the exposure to salt (NaCl 1% w/v) did not vary from heat tolerance without the salt. Low concentration of salt could have a protective role against heat stress while high salt concentration lowers the thermo-tolerance. In our study these results presented also the response of VP2 and VP3 to pretreatment with KCl, CaCl₂, or oleic acid, in increase the thermotolerance. The highest value of thermo-tolerance was obtained with offshoot leaflets. It could be concluded that 58.5°C might be the maximum heat tolerance due to the sufficient hardening conditions in the field and available of thick cuticle layer. From the above results, this investigation provided for the first time an accurate determination for heat tolerance of the tissue culture regenerated plants cv. Ruzaiz that are distributed to date palm growers. There is a potential to increase the thermo-tolerance of VP2, VP3, or offshoots that could increase their survival under field conditions by spraying plants leaves with, KCl, CaCl₂ (0.2 M) or oleic acid (0.1 M). The results suggested the importance of studying the thermotolerance of VP2 plants at various durations. Also, there is a need to verify a protective role of low concentration of NaCl against heat stress.

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