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Physiological and Biochemical Variations in Seed Germination of Cowpea (*Vigna unguiculata* L. Walp) Cultivars

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Abstract: Germination, coefficient of velocity of germination (CVG), lipid and sugar compositions were determined in whole ungerminated seed of three cowpea cultivars. The cultivars were chosen for biochemical characteristics based on seed germination and coefficient of velocity of germination tests. Texas cream 40 was able to germinate at very high and low temperatures. Black Crowder demonstrated acceptable germination at high temperatures but negatively affected at low temperature. Mississippi Purple obtained low germination percent and CVG at all temperatures studied. The main sugars present in cowpea seed are sucrose, raffinose and stachyose. Sugar contents were affected by cultivar. Sugar compositions were higher in the cultivars with high percent germination and reduced in the cultivar with lower percent germination suggesting the use of sugar for seed germination process. The most abundant fatty acids in cowpea seed were palmitic acid [$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$; (16:0)], palmitoleic acid [$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$; (16:1)], stearic acid [$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$; (18:0)], oleic acid [$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$; (18:1)], linoleic acid [$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2(\text{CH}_2)_6\text{COOH}$; (18:2)], linolenic acid [$\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$; (18:3)] and arachidic acid [$\text{CH}_3(\text{CH}_2)_{18}\text{COOH}$; (20:0)]. The result shows that the long-chain fatty acid appears to be important in the cowpea seed germination process. Thus, the information provided by this research will facilitate future plant physiological and genetic studies of cowpea cultivars.

Key words: CVG, fatty acid, raffinose, seed germination, sucrose, stachyose

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp), an indigenous African annual legume, is commonly known as southern pea, blackeye pea, crowder pea, lubia, niebe, coupe or frijole, (Craufurd *et al.*, 1996; Hall *et al.*, 1997). It is a high protein (25%), fiber (6.3%) and low fat (1.9%) vegetable crop having good nutritional qualities (Ricardo, 1985). It is a chilling sensitive crop (Ehlers and Hall, 1977; Hall *et al.*, 1997) and is adapted to warm weather and humid conditions (Craufurd *et al.*, 1996; Hall *et al.*, 1997). Cowpea is cultivated for food (Hall *et al.*, 1997), silage, green manure for soil improvement, livestock feed and pasture. Due to its nutritional content, versatility, adaptability and high yield, cowpea is chosen by the United States National Aeronautical and Space Administration as one of the few crops to be tested for cultivation in the space station (Ehlers and Hall, 1997). Furthermore, it is one of the mandated crops by the International Institute of Tropical Agriculture (Smartt and Hymowitz, 1985; Quin, 1997; Fery, 2002). Cowpea is a major agronomic crop in the United States during the early part of the 20th century, with production peaking at 2.4 million ha in 1937 (Fery, 1990). By the early 1980s, however, annual cowpea production in the United States was estimated at 197,680 acres (Fery, 1981). Cowpea has long been valued in the southern United States as a vegetable crop and an

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extensive industry currently distributes fresh, canned, frozen and dry-pea products nationwide. Additionally, cowpea has long been a popular item with home gardeners throughout the south. There is a broad range of characteristics among cowpea cultivars grown for horticultural use in the United States (Fery, 1990, 2002; Islam *et al.*, 2006).

Lipid contents have been related to seed vigor in some seed (Perl *et al.*, 1987). Seed of broad bean (*Vicia faba* L.), evening primrose (*Oenothera lamarckiana* De Varies) and carrot (*Daucus carota* L.) showed a drop of approximately 20 to 30% in the lipid fraction when subjected to accelerated aging; however, in cucumber (*Cucumis sativus* L.), squash [*Praecitrullus fistulosus* (Stocks) Pang] and pea (*Pisum sativum* L.) seed, an increase in lipid content was observed. In pepper (*Capsicum annum* L.) seed, the lipid content did not change with seed vigor level, but noticeable changes in fatty acid composition are observed. In the labeling of the lipids present in pea seed, phospholipids (70%), triglycerides (20%), waxes (8%), monoglycerides (2%) and diglyceride are detected (Harwood and Stumpf, 1970). Free fatty acids are seldom detected. The most common fatty acids in the phospholipids are palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3). The longer chain fatty acids are more common in the wax fraction. The endogenous fatty acids present in the seed were not always related to those synthesized during germination (Harwood and Stumpf, 1970). In germinating seed, *de novo* synthesis of long chain fatty acids occurs at early stages of germination. Fatty acids including palmitic (C16:0), stearic (C18:0), oleic acid (C18:1), linoleic (C18:2) and linolenic (C18:3), have been detected in cotyledons and axes of water oak (*Quercus nigra* L.), white oak (*Quercus alba* L.), american muskwood [*Guarea guidonia* (L.) Sleumer and *Carapa guianensis* Aubl.], silver maple (*Acer saccharinum* L.) and red buckeye (*Aesculus pavia* L.) (Connor *et al.*, 1996; Connor and Bonner, 1998; Connor *et al.*, 1998; Connor *et al.*, 2000; Connor and Bonner, 2001). Significant changes in the amounts of individual fatty acids in the seed and the subsequent changes in the total fatty acid content have been reported in these species.

Seed vigor has also been associated with carbohydrate content. In corn (*Zea mays* L.), the decline of sucrose and raffinose contents during aging was closely correlated with the loss of ability to germinate and rate of germination (Bernal-Lugo and Leopold, 1992). Furthermore, the quantity of oligosaccharides larger than sucrose (C₁₂H₂₂O₁₁) decreased as the period of imbibition during the germination process increased (Koster and Leopold, 1988). As a chilling-sensitive crop, cowpea is affected during germination and early seedling development if planted late in the spring when temperatures are still low in subtropical areas. Good stand establishment is necessary to avoid loss of yield, thus it would be beneficial to plant a cultivar able to tolerate low temperatures. The objective for this research was to determine the germination, coefficient of velocity of germination, lipid and sugar compositions of ungerminated whole seed of different cowpea cultivars.

MATERIALS AND METHODS

Cultivars/Genotypes Used in this Study

Trials were carried out over two years using 25 cowpea cultivars/genotypes grown to initially screen for germination and coefficient of variability of germination tests (Islam *et al.*, 2005, 2006). Three cultivars were chosen from 25 for further study for biochemical characteristics such as Texas cream 40 which showed ability to germinate at very high (40°C) and low (10°C) temperature; Black Crowder which had acceptable high germination at 40°C, but reduced germination at 10°C and Mississippi Purple which exhibited lower germination at all temperatures tested (Islam *et al.*, 2005, 2006). The seeds were obtained from Native Seed Search, Tucson, AZ, USA. The experiment was conducted during 2000 to 2002 at the Mississippi State University, USA. To get seeds from the same lot, cultivars were grown in the same year, under a common set of environmental conditions, handled using the same harvesting/seed processing equipment and stored under the same conditions.

Seed Germination

Germination percentages of all cultivars were evaluated under the methods described by Islam *et al.* (2006), except for the number of seed used per replication and the inclusion of different temperatures. Seeds were germinated using the between-paper method, in which two sheets of germination paper (Anchor brown germination paper, Anchor Paper Co., St. Paul, Minnesota) were placed in the bottom of 48×50 cm trays and an additional sheet of the same paper was used to cover the seed. The paper sheets were previously saturated with 2.5 times their weight of water. Four replications of twenty seeds of each cultivar were randomly assigned to trays.

Coefficient of Velocity of Germination (CVG)

The same procedures and conditions as used in the germination study were used in CVG evaluation. The numbers of germinated seed were evaluated every day for a period of 8 day. The CVG was calculated using the formula proposed by Islam *et al.* (2006).

Extraction of Carbohydrates

Seed carbohydrates were extracted and identified following the methodology suggested by Connor and Sowa (2000). To determine the initial content of carbohydrates in cowpea seed, whole dry seed were ground using a handy mill machine and 0.5 g of flour were used for carbohydrate extraction. For extraction from the germinating seed, after radical protrusion before first leaf expansion, the seed were dissected into cotyledons and axes. Tissues from each cultivar germinated at each temperature were separately dried in a freeze-dry system (Laconic Freezone 4.5 LABCONCO Corporation, Kansas City, MO). After removing all moisture, the tissue samples were finely-ground using a mortar and pestle. A 0.2 to 0.5 g dry tissue sample was used for each carbohydrate extraction. The tissue samples were placed in test tubes with 10 mL of an 80% ethanol solution and then boiled in a 75°C water bath for 1 h. The samples were then passed through a piece of filter paper in filter funnel. Additional ethanol solution was used to rinse the samples. The extracts were poured into evaporation flasks and then rotor-evaporated to dryness in a flask-evaporator (Buchler Instruments, Fort Lee, NJ). The evaporation flasks were rinsed with 10 mL of distilled water and the samples were freeze-dried overnights in a stoppered vacuum flask attached to the freeze-dry apparatus. When all the moisture was removed from the flasks, the samples were dissolved in 1 mL of Trimethylsilylimidiazole (TMSI), then heated in a 75°C water bath for 30 min and aspirated to dryness. The samples were then resuspended in 2 mL of chloroform and stored refrigerated until analysis.

Analysis of Carbohydrates

The analysis of carbohydrates was performed in a Hewlett Packard (HP) gas chromatograph (GC) equipped with a flame ionization detector and using a 125-5037 DB-5 column (30 m length×0.53 mm ID and 0.5 µm film thickness) (J and W Scientific Folsom, CA). The program in the GC preset a detector and injector temperature of 230°C. An initial oven temperature of 210°C was held for 7 min and then increased 15°C min⁻¹ until a temperature of 270°C was reached and held for 25 min. The flow was kept at 1.76 mL min⁻¹. Data were collected and printed in a HP printer attached to the GC. A graph and numerical map with the respective percentage peak area of each sugar was obtained. The carbohydrates were identified by comparing with standards of pure sugars prepared in a similar manner to the tissue samples and injected in the GC.

Extraction of Lipids

Fatty acids in the seed were extracted and identified through a modification of the methodology suggested by Whitaker (1986). To determine the content of fatty acids in cowpea seed, whole dry seed

were ground using a glass homogenizer and pestle. For extractions from germinating seed, after radicle protrusion and before first leaf expansion, the seed were dissected into cotyledons and embryos. Two to five grams of each type of tissue from each cultivar germinated at each temperature were finely chopped and transferred to Pyrex culture tubes and were then covered with isopropanol using a pasteur pipette. Tubes were then boiled in a water bath for 3 to 5 min, removed from the water bath and allowed to cool at ambient temperature. The supernatant (isopropanol fraction), was transferred to another tube and then dried under nitrogen to prevent airoxidation. The crude remains of the extract were resuspended with approximately 2 mL of 2:1 chloroform: methanol. To prevent autooxidation, the supernatants were then aspirated under nitrogen atmosphere until dry. Three milliliter of chloroform and 1.5 mL of methanol were added to these fractions, followed by the addition of 1.5 mL of 0.85% sodium chloride. This wash procedure described by Folch *et al.* (1957) was used to remove non-lipid contaminants from the samples. The tubes were then sealed under nitrogen and centrifuged at 1500x g (510.638 rpm) for 5 min to obtain better separation. The pellet parts were removed with a pasteur pipette and dried under nitrogen. The residues were taken up in 1.5 mL of 2:1 chloroform: methanol. The extracts from sample were combined. A second addition of 1.5 mL of 0.85% sodium chloride and a second centrifugation at the 1500 g for 5 min was performed to improve separation. The pellets were then removed and dried under nitrogen. The remaining portions were taken up in 2 mL of chloroform.

Transesterification of Lipids

The individual polar fractions were dried under nitrogen at 40°C. The samples were then resuspended in 0.5 mL of chloroform followed by the addition of 0.5 mL of 0.6 N KOH in dry methanol. The tubes were sealed under nitrogen and placed on a rotator in the dark at room temperature for 2 h. After the addition of 0.5 mL of distilled water and 50 µL 6 N HCl, the Fatty Acid Methyl Esters (FAME) were recovered by extraction with 2 mL of hexane.

Analysis of Lipids

Fatty acids compositions were identified and analyzed as described by Whitaker (1986). Lipids were analyzed in a Varian 3300 Gas chromatograph (Gas Chromatography, Varian Associates, Sugarland, TX) equipped with a flame ionization detector and using a Supelcowax 10 fused-silica wide-bore capillary column (30 m length×0.53 mm I.D. and 1.0 µL film thickness) (Supelco, Inc., Bellefonte, PA). The program used established an injector temperature of 250°C and a detector temperature of 300°C. The initial column temperature was set at 190°C and a holding time of 3 min. The column temperature was increased 3°C min⁻¹ until the final temperature of 220°C was reached and a holding time of 20 min. Data were transferred to a Varian 4290 integrator (Varian Instrument Division, Walnutcreek, CA), where they were expressed as percentage (%) area under the peak. Identification of the FAME was achieved by comparing them with the standards of FAME mixture reference No. 625006, 625007 and 625009 (Alltech Association Inc., Deerfield, IL).

Statistics

Each experiment was arranged in a completely randomized design (CRD) with five replicates of 20 seed each for each cultivar at each temperature (for germination and CVG). The data were analyzed as a combined series of CRDs. Data were subjected to analysis of variance (ANOVA) using the general linear models (GLM) procedure of SAS version 8.1 (SAS Instit., Inc. Cary, North Carolina, USA). Mean separations were done using Fisher's protected least significant difference (LSD) test.

RESULTS AND DISCUSSION

Seed Germination and CVG

The germination percent and coefficient of velocity of germination (CVG) were demonstrated in Table 1. Usually one of the suitable tests to describe seed vigor is the CVG and this test combines the germination percentage and rate of germination into a single equation (Islam *et al.*, 2006). The three cowpea cultivars were chosen for biochemical evaluation based on physiological performance of the seed namely performance in the germination and CVG tests under temperature stress conditions (Islam *et al.*, 2005, 2006). Texas cream 40 which performed well at 10, 30 and 40°C temperatures; Black Crowder which was more sensitive to chilling temperature than high temperature (Islam *et al.*, 2005, 2006) and Mississippi Purple with low germination and CVG at all temperatures such as 10, 30 and 40°C (Islam *et al.*, 2005, 2006).

Sugar Compositions

Sugar compositions namely sucrose, raffinose and stachyose content were significantly differed among the cultivars (Table 2). Black Crowder had the highest sucrose, raffinose and stachyose contents. This cultivar also exhibited high germination percentage. The lowest content of sucrose, raffinose and stachyose was in Mississippi Purple, which had low germination percentage. The sucrose content in ungerminated seed was highest in Black Crowder, followed by Texas cream 40 and lowest in Mississippi Purple. Sucrose is a primary carbon source in cells of higher plants to support as energy resources (Xu *et al.*, 1989). During seed development changes in carbohydrates occur in such way that monosaccharide content decreases whereas oligosaccharide content increases, but during the germination processes this pattern is reversed (Vertucci and Farrant, 1995). In this study, Black Crowder seed had faster and higher percent germination than Texas cream and Mississippi Purple; thus, the higher sucrose content in the above cultivar suggest faster and more efficient use of sucrose in germination process and seedling development in this cultivar. Xu *et al.* (1989) indicated that during germination seed have a dual role: first as a source of sucrose produced in cotyledons and secondly as sink when the young seedling uses sucrose for development. The highest raffinose content mean was in black crowder (2.46 mg g⁻¹) and lowest content in Texas cream 40 (1.53 mg g⁻¹) and Mississippi Purple (1.25 mg g⁻¹). The highest stachyose content mean in the whole ungerminated seed was in black crowder (10.54 mg g⁻¹), followed by Texas cream 40 (7.78 mg g⁻¹) and Mississippi Purple (5.77 mg g⁻¹). There were a significant (p<0.01) positive correlation between sugar contents and

Table 1: Germination percentage and coefficient of velocity of germination (CVG) of three selected cowpea cultivars evaluated at different temperatures

Name of varieties	Germination (%) ^z	CVG ^z
Mississippi purple	63.30±0.65 ^b	0.151±0.00008 ^a
Texas cream-40	93.80±0.76 ^a	0.155±0.00009 ^a
Black crowder	94.20±0.70 ^a	0.154±0.00012 ^a
LSD (0.05)	9.27	0.012

Data are means of five replications±standard error. ^z: Analysis performed on arcsine transformed data. ^a: Means within a column not followed by a common letter are significantly (p<0.05) different according to Fisher's protected LSD test

Table 2: Sugar compositions of the three cowpea cultivars

Name of the varieties	Sugar profiles		
	Sucrose content (mg g ⁻¹)	Raffinose content (mg g ⁻¹)	Stachyose content (mg g ⁻¹)
Mississippi purple	9.97 ^{c*}	1.25 ^b	5.77 ^c
Texas cream 40	14.81 ^b	1.53 ^b	7.78 ^b
Black crowder	18.31 ^a	2.46 ^a	10.54 ^a
LSD (0.05)	2.06	0.31	1.51

*: Means within a column not followed by a common letter(s) are significantly (p<0.05) different according to Fisher's protected LSD test

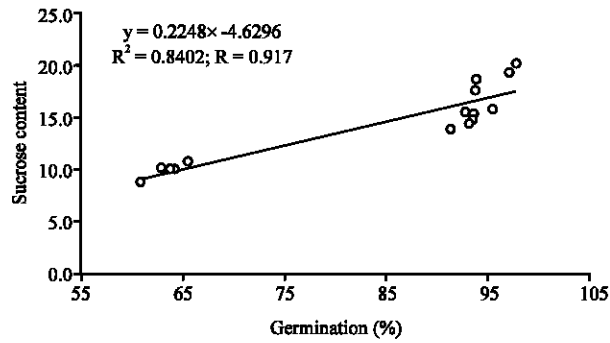


Fig. 1a: Relationship between germination (%) and sucrose content of cowpea seed

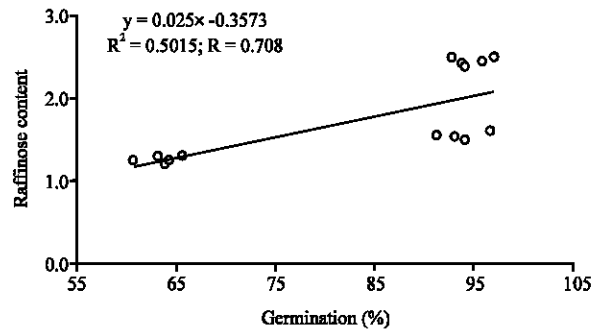


Fig. 1b: Relationship between germination (%) and raffinose content of cowpea seed

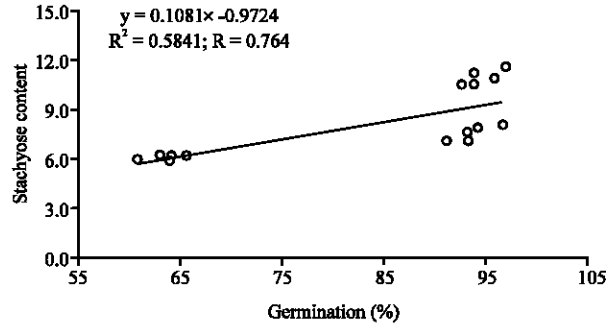


Fig. 1c: Relationship between germination (%) and stachyose content of cowpea seed

germination percentage of cowpea seed. Sucrose has the most intimate relationship ($R = 0.917$) with the seed germination percentage of cowpea seeds followed by stachyose ($R = 0.764$) and raffinose ($R = 0.708$) (Fig. 1a-c). The results indicate that sucrose *de novo* synthesis was higher in cowpea seeds and it played a vital role in cowpea seed germination process. Koster and Leopold (1988) demonstrated that raffinose as well as stachyose plays an important role in desiccation tolerance of pea, corn and soybean seed using sucrose crystallization. The sugar accumulation in the ungerminated seed and in cotyledon tissues of germinated seed at low temperature indicated either *de novo* synthesis or convection of other products to sucrose. Miguel and Browse (1995) pointed out that seed storage components such as lipids, proteins and carbohydrates are converted to sucrose in order to support embryo development.

Table 3: Lipid profiles of three cowpea cultivars

Name of the varieties	Lipid profiles (%)						
	Palmitic acid (16:0)	Palmitoleic acid (16:1)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)	Arachidic acid (20:0)
Mississippi purple	31.60 ^{a*}	Traces	3.44 ^b	6.13 ^b	37.71 ^b	18.96 ^b	1.97 ^a
Texas cream 40	27.09 ^b	0.20 ^a	5.09 ^a	7.09 ^a	35.98 ^a	23.19 ^a	1.05 ^b
Black crowder	26.59 ^b	Traces	4.08 ^b	4.43 ^c	40.35 ^a	22.82 ^a	1.44 ^{ab}
LSD (0.05)	2.22	0.12	0.80	0.54	1.03	0.74	0.80

*: Means within a column not followed by a common letter(s) are significantly ($p < 0.05$) different according to Fisher's protected LSD test

Table 4: Ratio of the different fatty acids in three cowpea cultivars

Name of the varieties	Ratio of linoleic acid (18:2) to oleic acid (18:1)	Ratio of 18-carbon unsaturated to 18-carbon saturated fatty acid	Ratio of unsaturated to saturated fatty acid
Mississippi purple	6.18 ^{b*}	18.35 ^a	1.80 ^b
Texas cream 40	5.08 ^c	13.24 ^b	2.05 ^a
Black crowder	9.14 ^a	16.58 ^a	2.21 ^a
LSD (0.05)	0.67	2.21	0.17

*: Means within a column not followed by a common letter(s) are significantly ($p < 0.05$) different according to Fisher's protected LSD test

Lipid Compositions

The main fatty acids present in cowpea seed and seedlings were palmitic acid (C16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3) and arachidic acid (20:0). The lipid compositions of the cowpea cultivars were presented in Table 3. The palmitic acid (16:0) was highest in Mississippi Purple. This cultivar had a lower germination percentage, suggesting that palmitic acid was not essential to the cowpea seed germination process. Palmitoleic acid (16:1) was extracted in small quantity in the seed of Texas cream 40. Stearic acid (18:0) was highest in the seed of Texas cream 40 which was highest germination percentage, while Mississippi Purple was lowest and showed a low germination. Thus this long-chain fatty acid would appear to be important in germination process. Oleic acid (18:1) was highest in cream 40, the cultivar with high germination. Linoleic acid (18:2) was highest in the cultivar with high germination. Linolenic acid (18:3) was higher in the cowpea seed of higher germination (Texas cream 40 and Black Crowder) and lower in Mississippi Purple. Arachidic acid (20:0) was higher in both cultivars with high and low germination [Mississippi Purple (a) and Black Crowder (ab) (Table 3)]. Harwood and Stumpf (1970) pointed out that the initial production of fatty acid in germinating pea seed showed a lag which depended on temperature. They also observed that production of fatty acids during the first 30 h of germination initiated with palmitate and stearate, followed later by oleic acid. Polyunsaturated acids were undetected during this period of pea seed germination. Stearic acid is found in fatty acid composition of important oilseed plants such as soybean (*Glycine max* L. Merr.), rapeseed (*Brassica napus* L.), sunflower (*H. annuus* L.), palm oil (*Elais guineensis* L.), cotton (*G. hirsutum* L.), peanut (*A. hypogaea* L.) and coconut (*Cocos nucifera* L.) in proportions of approximately 4% (Miguel and Browse, 1995). Thus, this long-chain fatty acid appears to be important in germination process.

Ratio of Fatty Acids

Table 4 shows the ratio of fatty acid composition in the seed of three cowpea cultivars. The C18:2/C18:1 ratio was higher in Black Crowder followed by Mississippi Purple and Texas cream 40. The 18-carbon unsaturated to 18-carbon saturated fatty acid ratio was lower in Texas cream 40 than in Black Crowder and Mississippi Purple. The ratios of unsaturated to saturated fatty acids were higher in Black Crowder and in Texas cream 40 than in Mississippi Purple (Table 4). Miguel and Browse (1995) reported that long-chain fatty acids are common in seed from the *Brassicaceae* and other species such as meadowfoam (*Limnanthes alba* Benth) and jojoba (*Simmondsia chinensis* L.).

Membrane lipids contain mainly unsaturated fatty acids such as 18:2 (Linoleic acid) and 18:3 (Linolenic acid). When plants or plant parts are exposed to low temperature, the desaturation of fatty acids occurs mainly from 18:2 to 18:3 (Murata and Los, 1997). The importance of the desaturation of fatty acids of membrane lipids in tolerance to low temperatures has been demonstrated in transgenic systems. When relative levels of saturated molecular species of phosphatidylglycerol are reduced by transformation with glycerol-3-phosphate acyltransferase from a chilling resistant plant, plants become more tolerant to low temperatures (Murata and Los, 1997). The presence and composition of fatty acids in the seed affected its ability to germination procedures. The differences in germination capacity may be related to over expression or inhibition of genes encoding synthesis of relevant molecules that reflect the composition differences demonstrated in this study (Khan and Ungar, 1997; Castellon *et al.*, 2003). These changes could be explained by the possible translocation of fat-like substances from reserves (cotyledons) to the sink where the respiratory process is more active, or due to lipid peroxidation.

CONCLUSIONS

From the above results and discussion it is apparent that the main sugars present in cowpea seed were sucrose, raffinose and stachyose. The cowpea cultivars with high germination percentage showed higher sucrose, raffinose and stachyose contents in the seed. The differences in germination capacity may be related to over expression or inhibition of genes encoding synthesis of relevant molecules that reflect the composition differences. These changes could be explained by the possible translocation of fat-like substances from reserves to the sink where the respiratory process is more active. The results may help in future breeding for specific constituents to enhance or reduce the fatty acid and sugar contents and for improvements of the desired quality criteria of cowpea seeds.

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