International Journal of Academic Research and Development
ISSN: 2455-4197, Impact Factor: RJIF 5.22
www.academicsjournal.com
Volume 1; Issue 7; July 2016; Page No. 37-39

Amino acids profiles of two coconut (*Cocos nucifera*) progenies as influenced by breaking of primary dormancy

[1] William Ubi, *[2] Godwin Michael Ubi, [3] Martina W. Ubi, [4] Thomas Okweche

[1, 4] Department of Science and Technology, National Open University of Nigeria, Calabar Study Centre, Nigeria
[2] Department of Genetics and Biotechnology. University of Calabar, Nigeria
[3] Cross River State Agricultural Development Programme CRADP, Calabar, Nigeria

Abstract
Experiment was carried out in 2015 and 2016 to evaluate changes in Amino Acids during breaking of dormancy in two coconut (*Cocos nucifera*) progenies. The experiment was carried out using completely randomized design at Emmanuel College Laboratory Idomi, Cross River State, Southern Nigeria. The results showed that the kinds of Amino Acids and their amounts varied significantly ($p<0.05$) and their co-efficient of variability in most cases was 24%. The values obtained in the amount of Amino Acids in endosperm of dominant and non-dominant coconut with co-efficient of variability of 5% were lower than mean values obtained from the embryo. During release from dormancy, the amounts of various Amino Acids increased in both the embryo and endosperm. In the embryos, the greatest increases were in alanine, glutamic acid, proline, serine and argemine. In the endosperm the increase during dormancy release were greatest in alanine, glutamic acid, proline, serine and valine. Variations in amount of amino acids among progenies was recorded which of course was due to differences in the degree of seed dormancy. The amount of amino acids in both dormant and non-dormant embryos varied significantly ($p<0.05$) among progenies. These differences were much greater for non-dormant than for dormant embryos. These results are discussed in light of changes in amino acid during breaking of dormancy.

Keywords: Dormancy breaking, amino acid contents, embryo, endosperms, *Cocos nucifera*

Introduction
During the actual germination process of seeds from dormancy, various aspects of metabolism have been studied for a variety of plant species. Carbohydrate and lipid relations and some aspects of protein metabolism have been investigated in detail on seed germination (Kollar *et al.,* 1962; Kozlowski, 1971-1972; Mayer and Poljakoff-Mayber, 1975) [7].

Studies during seed germination indicate that nitrogen metabolism occurs (Williams and Ross, 1973). As reserve proteins are degraded, there is an increase in the amount of amino acids and amides followed by synthesis of new proteins in the growing embryo part of the seed (Durzon *et al.,* 1971; Koller *et al.,* 1998) [5]. New proteins apparently formed are from the nitrogen pool resulting from protein degradation. Coconut, botanically called *Cocos nucifera* is an early Spanish crop which explorers called *Cocos* meaning "monkey face", because the tree indentation (eye) on the hairy nut resembles the head of a monkey, *nucifera* means nut-bearing coconut provides a nutritious source of meat, juice, milk and oil that has fed and nourished populations around the world for generations (Stokes, 1988; Pack, 1989) [13, 9]. On many Islands, coconut is in the diet and provides the majority of the food eaten. Nearly one-third of the world's population depends on coconut to some degree for their economy (Stock, 1965). Coconut is rich in fibre, vitamins and minerals together with amino acid (Fine and Barton, 1998) [3]. Coconut is used worldwide to treat a wide variety of health problems, thus, it is used as food and medicine. Relatively, little research has been carried out on amino acid changes during release from seed dormancy as distinct from changes occurring during the actual germination process. This study was to examine the amino acid changes in the embryo and endosperm during heat induced breaking of dormancy of coconut (*Cocos nucifera*)

Materials and Methods
Two coconut seeds, a hybrid and a local cultivar were maintained at ambient temperature (20-25 ^{o}C). Dormancy was broken by a 35 day exposure at 42 ^{o}C in an incubator (Odetola, 1974).

Extraction
Air-dried dormant and non-dormant nuts were broken and embryos were removed from the kernels. Excised embryos were then rapidly crushed and ground in a mill. Sixty grammes of milled kernel were placed in a beaker containing 200ml of boiling 95% ethanol, and allowed to simmer for 30mins. The endosperm was extracted as follows: the ethanol was decanted while the residue was thoroughly homogenized intermittently in a warring blender at high speed for 20mins. The homogenate was centrifuged at 2000 rpm for 15mins. The supernatant was decanted and the residue re-extracted by suspension in 50ml of 80% ethanol and gentle boiling for 15mins. The residue was then centrifuged and the supernatant combined with that obtained earlier.

The re-extraction process was repeated and all supernatants were combined with that from the 95% ethanol previously obtained in the initial step. The procedure for embryo extraction was similar except that instead of homogenizing in a blender, embryos were ground to a fine powder in a mortar and pestle. One hundred ml of distilled water was added to the combined extract and defatted by shaking gently twice with a

total of 100ml, 60-80 ^{o}C Bp petroleum ether. The lower aqueous fraction in the separator funnel was concentrated by evaporation on a hot plate at low temperature.

Purification and Analysis

A small amount of cation exchange resin (Dowex 50) was purified as follows: the resin was placed in a beaker and the floating portion was separated and discarded. The resin was then packed on a sephadex column and purified by washing first with 40ml of 4% NaOH and rewashing twice with total of 100ml of deionizer water. To convert the resin to the acid form, 50ml of 2NHCL acid was added. To remove excess acid the resin was washed with a total of 100ml of deionized water. The extract was adjusted to pH 3.0 with 2NHCL, and poured onto the column. The rate of flow of extract through the column was controlled by adjusting the air pressure applied to the top of the column. The column was washed twice with 50ml portion of deionized water. Fifty milliliters of 4% NH_4OH was then poured onto column. The resin turned bluish on contact with the ammonia solution and movement of the blue front down the column was followed until it reached the bottom. The effluent was collected and the column washed with 50ml of deionized water and combined with that obtained earlier. The combined effluent was evaporated to dryness in arotary evaporator at 60 ^{o}C. The residue was taken up with a small volume of 20% ethanol and re-evaporated to dryness. The residue was re-dissolved in a minimum volume of 15% isopropanol and re-evaporated gently to dryness. The residue was taken up with citrate buffer at a pH of 2.5 and made up with buffer to 1ml. Quantities of amino acid in samples was determined with Beckman Autoanalyzer, model 120B, following the procedure of Kemp *et al.* (1972).

Results

The kind and amounts of various amino acids in both embryo and endosperm varied appreciably among the hybrids, (Table 1). Following the breaking of dormancy, there was a general and consistent increase in the amounts of free amino acid in the embryo of the hybrids.

Table 1: Amount of free Amino Acids (µ mole/g) in embryos of dormant and non-dormant coconut seeds

Amino acid	Exotic Hybrid seed		Hybrid Local seed	
	Dormant	Non-dormant	Dormant	Non-dormant
Glycine	0.21	0.82	0.14	0.69
Aspartic acid	1.12	3.78	0.85	2.92
Threonine	0.35	1.29	0.15	0.84
Serine	1.31	5.92	0.26	1.38
Proline	0.75	5.63	0.12	0.52
Glutaminc acid	3.48	8.46	1.05	2.16
Alamine	2.72	13.44	0.44	2.32
Valine	0.52	2.06	0.18	0.75
Isoleucine	0.46	0.85	0.17	0.68
Leucine	0.24	0.78	0.13	0.34
Tyrosine	0.34	0.94	0.15	0.36
Phenylalamine	0.18	0.32	0.14	0.16
Lysine	0.26	2.55	0.17	1.33
Histidine	0.19	3.31	0.16	2.18
Arginine	1.17	6.30	0.15	3.19
Tryptophan	0.16	0.29	0.13	0.18
LSD (0.05)	0.03	0.05	0.03	0.04
SE	0.02			
CV	24%			

In particular area aspartic acid, alarnine, lysine, glutamic acid and arginine increased markedly. Equally, a significant amount of amino acids that were present in traces in the embryo were found in appreciable amounts after dormancy was broken. These included proline, glycine, histidine, isoleucine and valine and the trend was common in both exotic and local hybrids. The amounts of amino acids generally were much lower in the endosperm than in the embryo, both before and after dormancy was broken and their differences was statistically significant (p<0.0) (Table 1). Protein crystals similar to those found in other oily seeds were found in coconut endosperm. These crystals became rearranged and grouped during breaking of dormancy with a gradient of rearrangement from the embryo towards the coat. The growing of these crystals was prelude to their utilization. After dormancy was broken, there was a general increase in the amount of free amino acid component of the endosperm. The largest increases were in alamine, valine, glutamic acid, serine and aspartic acid. As was the case with embryo, the amino acids that were not detected in the fresh endosperm appeared after dormancy was broken. During progressive release from dormancy, the dry weight of the endosperm decreased and cells adjacent to embryo eventually collapsed.

Table 2: Amount of Amino Acids (µ mole/g) in endosperm of dormant and non-dormant coconut hybrid seeds

Amino acid	Exotic Hybrid seed		Hybrid Local seed	
	Dormant	Non-dormant	Dormant	Non-dormant
Aspartic acid	0.02	0.04	0.02	0.03
Threonine	0.03	0.05	0.02	0.03
Serine	0.64	2.05	0.03	0.04
Proline	0.43	3.03	0.03	0.05
Glutaminc acid	0.24	2.06	0.03	0.06
Glycine	0.03	0.05	0.002	0.05
Alamine	0.04	0.08	0.02	0.06
Valine	0.68	3.18	0.02	0.06
Methionine	0.53	3.04	0.02	0.03
Isoleucine	0.02	0.03	0.02	0.03
Leucine	0.03	0.04	0.03	0.04
Tyrosine	0.03	0.05	0.03	0.06
Phenylalamine	0.02	0.04	0.02	0.04
Lysine	0.04	0.06	0.03	0.05
Histidine	0.03	0.05	0.02	0.04
Arginine	0.02	0.04	0.02	0.04
Tryptophan	0.02	0.03	0.02	0.04
LSD (0.05)	0.002	0.003	0.002	0.003
SE	0.002			
CV	5%			

Whereas the amount of arginine and aspartic acid increased greatly in the embryo on breaking of dormancy, (Table 2). They increased only slightly in the endosperm. Some of these are aspartic acid, glycine, methionine, leucine, phenylalanine, histidine, argentine and tryptophan appearing in both hybrids. It appears that disintegration and hydrolysis of protein crystals are associated with changes in amino acids.

Discussion

After dormancy of the coconut seeds were broken, the same sixteen amino acids were found in the embryo and endosperm. The amount of amino acids were significantly (p<0.05) higher in the embryo than in the endosperm, both in dormant and non-dormant seeds (Ruven, 1996; Singh, 2001) [10, 11]. In both hybrids, the amount of individual amino acids increased in both the embryo and endosperm after dormancy was broken, probably as a result of protein hydrolysis (Khoo and Wolf, 2000; Koller *et al.,* 1998) [5, 4]. The largest increases were in alamine when the seeds were non-dormant in the exotic hybrid. As a matter of fact, the rolls of amino acids during and shortly after release of seed dormancy in coconut are not fully understood. Ruven (1956) suggested that glutanic acid, a precursor of glutamine was required to maintain normal plant growth. Stewart *et al.* (1994) [12] reported that glutamine and glutamic acid were functionally related to protein synthesis and metabolism in young meristems, whereas asparagines were important only in regions where growth has ceased (Fine and Barton, 1958). It was suggested that increases in glutamic acid and aspartic acid during maturity of coconut seed could be associated with their use as precursors of other amino acids (Koziloweki, 1972). The impertinence of amino acids in germination processes is supported by studies with applications of amino acids to dormant seeds. For instance, Stone and Smith (1991) [15] reported that seed germination was stimulated by applications of asparagine and leucine to seed showing low germination capacity. The much greater amount of amino acids in the embryo than in the endosperm may be explained by absorption of protein by the haustorium prior to hydrolysis or by mobilization of hydrolyzed protein by the embryo from the endosperm (Webster, 2003) [16]. Little variations among the hybrids of coconut in the amount of amino acid may be due to differences in the degree of seed dormancy (Adams and Novellie, 1975; Stocks, 1993) [1, 14].

This view is reinforced by the fact that the amount of heat required to break seed dormancy varies among hybrid and is a subject of further investigation of protein and amino acid metabolism in dormant and non-dormant coconut seeds.

Conclusion

The results of this investigation has shown that the amount of amino acids in both dormant and non-dormant embryos varied appreciably among progenies, though these differences was much greater for non-dormant than for the dormant embryos. Variations in the amount of amino acids among progenies may be due to the differences in the degree of primary seed dormancy. Protein crystals similar to those found in other oil seeds was also found the coconut endosperm. These crystals became rearranged and grouped during the breaking of primary dormancy. Coconut seeds are useful for further investigation for protein and amino acids metabolism in the dormant and non-dormant coonut seeds.

References

1. Adams CA, Novellie L. Acid hydrolases and autolytic, properties of protein bodies and spherosomes isolated from undermunated seeds of sorghum bicolor (Linn). Moench. Plant Phy. 1975; 55:7-11.
2. Durxan DK, Mia AJ, Ramalah PK. Metabolism and sub-cellular organization of the jack pine embryo (*Pinus banksiana*) during germination. Can. J. Bot. 1971; 94:927-938.
3. Fine J, Barton LV. Biochemical studies of dormancy and after ripening in seeds. 1-Changes in Free Amino Acid Content Contr. Boyce Thompson Inst. 1989; 18:438-500.
4. Khoo U, Wolf MJ. Origin and development of protein granules in maize endosperm. Am. J. Bot. 2000; 57:1042-1050.
5. Koller D, Mayer AM, Poljakoff A, Kleins. Rev. Plant. Physiol, 1998; 13:437-464.
6. Koziloweki TT. Seed Biology Academic Press, New York, 11.
7. Mayer AM, Poliakoff A. The germination and seedling development of juniper seeds. Pergamon Press, New York, 1975.
8. Odetola JA. Heat requirement of oil palm seeds for germination. 1 Relation of Seed Age to Heat Requirement. J. Niger Inst. Oil Palm Res. 1974; 5:79-84.
9. Pack DA. Chemistry of after-ripening germination and seedling development of juniper seeds. Bot. Gaz. 1989; 72:139-150.
10. Ruven AH. Glutamine and asparagin as nitrogen sources for the growth of plant embryos in vitro: A comparative study of 12 species. Austral. J. Biol. Sci. 1996; 9:511-527.
11. Singh J. The isolation and partial characterization of arachin from seeds of Arachis hypogarea Ph.D. Dissertation Taxas 5.M. University, 2001, 6.
12. Steward FC, Wetmore R, Thompson J, Nitsch JP. A quantitative chromatographic study of nitrogenous components of shoot species. Amer. J. Bot. 1994; 41:123-134.
13. Stokes P. Temperature and seed dormancy. Encycl. Plant Physiol. 1988; Xv(12):746-803.
14. Stokes P. The stimulation of growth by low temperature in embryo of Heracleum sphondylium I. J. Exp. Bot. 1993; 4:222-234.
15. Stone GE, Smith R. Influence of chemical solution upon the germination of seeds. Rep. Massachusetts Agric Exp. Station, 1991; 13:74-83.
16. Webser OC. Nitrogen metabolism. Arn. Rev. Plant Physiol. 2003; 9:245-280.