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GENETIC VARIATION OF SEED PHYSICO-CHEMICAL PROPERTIES OF *GONGRONEMA LATIFOLIA* ACCESSIONS IN RELATION TO THEIR SEEDLING PERFORMANCE

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ABSTRACT

Genetic variation in physico-chemical properties of different accessions of *Gongronema latifolia* and contributions of such traits to seed emergence were investigated for two years in Nsukka. The study showed significant differences in the studied traits of the *G. latifolia* accessions. The accession, AC-05 had larger seeds as well as higher seed breadth and cotyledon thickness but lower level of abscisic acid (ABA) in both years. The seedling emergence capacity of the accessions in both years showed that the seedlings emerged earlier and more vigorous in the second year with reduced moisture content and ABA. Higher level of genotypic and phenotypic variance among the accessions on the measured traits occurred on the phenol content. Heritability estimates were higher in the chemical traits of the seeds than the physical traits with the exception of seed weight. Furthermore, the correlation coefficients suggest that accessions with larger seeds also have lower ABA and significantly higher vigorous emergence suggesting the impact of genetic factors in determining the performance of accessions. It was concluded that different accessions showed genetic differences in the seed traits which directly influenced emergence rate and vigour of seedlings over the two years.

Key words: Genetic variation, seed traits, emergence, abscisic acid, *Gongronema latifolia.*

INTRODUCTION

Gongronema latifolia Benth is one of the widely used species of the genus in sub-Saharan Africa and some parts of China (Nielsen, 1965; Ying and Ping-tao, 1977). It is of the Asclepiadaceae family and is called utazi and arokeke in Southeastern and Western Nigeria, respectively. The plant is used as a leafy vegetable and is a good source of vitamins, protein, iron and minerals (Okafor, 1997). Agbo *et al.* (2009) and Eleyinni (2007) had reported as high as 58 and 27% levels of protein in the species respectively. The plant is traditionally useful in the treatment of cough, malaria, worm infections, constipation, throat pain and typhoid fever in Nigeria (Agbo *et al.,* 2005; Okafor, 1997). Burkhill (1985) had earlier shown that the plant is in use for new born babies and children in Sierra Leone and Ghana.

Production of the plant (that had been harvested from the wild) in a regular culture is recently gaining attention as it has been shown that it can be propagated by stem cuttings as well as by seeds (Agbo and Omaliko, 2006; Agbo and Obi, 2008). Seeds of the species are relatively small ranging from 7 mm to 13 mm in length and 3 mm to 8 mm in diameter (thickest part).

Physical and chemical properties of seeds of crop species have been shown to influence their germination and emergence (Yogeesha *et al.,* 2006; Raven *et al.,* 2005; Batistella *et al.,* 2002). Diversity in physico-chemical properties of seeds of accessions of *G. latifolia* may arise as a result of genetic differences as reported in traits of other crops (Hamrick and Godt, 1996; Ellison *et al.,* 2004). A preliminary investigation showed differential emergence rates in different accessions of *G. latifolia* species. The differences in the emergence rates are a function of the seed quality. Seed quality is normally determined by a number of physical and chemical properties of seeds that confer genetic differences in varieties within species (Keefe, 1999; Batistella *et al.,* 2002). Such genetic variation involve differences between two or more genetic lines. There is paucity of information on the levels of variation in seeds of different accessions of *G. latifolia* in relation to their emergence rates. Hence, the study was designed to understand the level of genetic variation in physical and chemical properties of seeds of different accessions of *G. latifolia* and the contributions of such properties to seed emergence.

MATERIALS AND METHODS

Stem cuttings of different accessions of *G. latifolia* were obtained from different localities of southeastern Nigeria in 2006. The cuttings were used to establish the accessions in the Department of Crop Science, research farm, University of Nigeria, Nsukka, Nigeria. Thirty stands of each accession were planted in three rows in a plot at a spacing of 1m x 1m between and within rows. The field was laid out in a randomized complete block design with three replications and was manured with poultry droppings at the rate of 20 tons/ha.

All other cultural practices including regular weeding and staking were undertaken. By the second and third year after establishment, 2008 and 2009, twenty follicles containing seeds from each accession were picked at the yellow stage (dehiscent line just shown) and used for physico-chemical evaluation and emergence tests. The yellow stage was reported as the optimum time to pick the follicles as it preserves seed losses as well as gives higher germination rates (Agbo and Obi, 2008). The follicles containing the seeds were stored in the laboratory bench of Department of Crop Science, University of Nigeria, Nsukka. The physical traits measured included seed length, seed breadth, seed weight and cotyledon thickness. Seed length and breadth were measured with a metric ruler, seed weight and cotyledon thickness was measured with sensitive weighing balance and vernier calipers, respectively. Some chemical contents of the seeds determined included: protein, phenol, abscisic acid, fat and moisture. The chemical constituents of the seeds were determined following the official methods of AOAC (1980).

The physico-chemical and emergence tests of the seeds were done a week and two weeks after picking of follicles in 2008 and 2009, respectively. Nursery beds measuring 120 cm x 30 cm were prepared from a previously plowed and harrowed field. A decomposed poultry manure was applied on the beds at the rate of 20 tons/ha. The beds were spaced 50 cm x 50 cm and the treatments were arranged in a completely randomized design (CRD) with five replications. The experimental site was under 65% shading to create a homogeneous environment. Fifty seeds were sown on each bed according to the assigned accession. The beds were watered regularly. Emergence of seedlings recorded daily was noted as the first appearance of any portion of the seedling above the soil surface. The following traits were calculated from emergence records: cumulative emergence percentage (CEP), cumulative dormancy period (CDP), mean daily germination (MDG) and rapidity of emergence which was estimated by a coefficient of velocity of emergence (C.V.E.) after Kotowski (1978) as,

$$C.V.E. = 100 \frac{x \quad A_1 + A_2 + \cdots + A_x}{A_1T_1 + A_2T_2 + \cdots + A_xT_x}$$

where A = number of seedlings emerging and

T = number of days after sowing.

Data were collected in three replicates for the chemical constituents and five replicates for emergence tests per accession for each of the two years. Data were analyzed with GENSTAT Discovery Edition 3.0 (2007).Duncan's new multiple range test was used to separate the means that differed significantly (Gomez and Gomez, 1984)

RESULTS

The physico-chemical characteristics of the *G. latifolia* accessions used are presented in Tables 1 and 2. The accession, AC–05 had the highest seed breadth (BTH) and was significantly ($p < 0.05$) higher in cotyledon thickness when compared to other accessions in both years. AC–03 had significantly smaller cotyledon thickness in both years. On the other hand, AC–04 had significantly ($p < 0.01$) higher seed length. The moisture contents of all the accessions were lower in the second year when they were exposed for fourteen days before use for emergence tests as well as for physico-chemical characteristic determination. AC–04 and AC–05 had significantly higher moisture (MC) and lower abscisic acid (ABA) contents in both years. Similarly, AC–04 had significantly higher level of phenol in both years. The seedling emergence capacity of the accessions is shown in Table 3. It showed that the seedlings emerged earlier and were more rapid in the second year with reduced moisture contents of the seeds than in the previous year. AC–05 that had the highest cotyledon thickness and lowest level of abscisic acid had significantly ($p < 0.05$) higher seedling emergence percentage of 98 in both years. On the other hand, AC–03 with the lowest cotyledon thickness and highest level of abscisic acid had significantly lower emergence percentages of 55 and 62, respectively, in both years.

The correlation coefficients of the measured seeds and seedling traits showed that abscisic acid content of the seeds was significantly and negatively correlated to cumulative emergence percentage (CEP), cotyledon thickness, moisture content and mean daily germination (MDG) (r = -0.76, -0.57, -0.81, -0.69, respectively; $n = 36$) (Table 4). Conversely, CEP was significantly ($p < 0.05$) and positively related with CTH, LTH, MC, MDG and phenol (r = 0.73, 0.55, 0.93, 0.94 and 0.53, respectively; $n = 36$). Phenolic contents of seeds showed significant ($p < 0.05$) and positive correlation with CVE, MC and MDG (r = 0.57, 0.64 and 0.59 respectively; $n = 36$). Protein content of the seeds on the other hand, showed negative and non-significant relationship with all the measured traits of the seeds and germination parameters with the exception of CVE and MDG.

The variance estimates of the physico-chemical traits of the *G. latifolia* accessions for the two years are presented in Table 5. There was high genetic variance in fat and cumulative emergence percentage in the accessions in both years. Cotyledon thickness and 100-seed weight showed low levels of genetic variation in the years. Higher genotypic coefficient of variation was observed in phenol contents of the seeds. The chemical components of the seeds had higher broad sense heritability estimate than the physical traits in both years.

Table 1. Physico-chemical composition of the six accessions of *G. latifolia in 2008.*

Accessions	Physical Characteristics				Chemical Characteristics				
	BTH (mm)	CTH (mm)	LTH (mm)	100-seed weight (g)	ABA (%)	FAT (%)	Phenol (mg/100g)	Protein (%)	MC (%)
AC-01	0.39^{a}	0.72^{b}	0.87^{c}	0.40^{d}	20^{a}	13^{b}	0.7^{c}	18^{c}	24c
AC-02	0.39^{a}	0.82^{a}	0.86^{c}	0.42^{c}	20^{a}	15^{b}	0.7^{c}	18^{c}	24^{c}
AC-03	0.40^{a}	0.59^{c}	0.85^{c}	0.31^{f}	22^{a}	6^{d}	1.9^{b}	21^{b}	20^{d}
AC-04	0.40^{a}	0.82^{a}	0.97^{a}	0.45^{b}	15^{c}	18^{a}	2.3^{a}	18^{c}	31^{b}
AC-05	0.42^{a}	0.87^{a}	0.91^{b}	0.52^{a}	11^{d}	11^{c}	1.9^{b}	20^{b}	33^{a}
AC-06	0.38^{a}	0.61^{c}	0.80^{d}	0.36^{e}	18^{b}	15^{b}	0.8^{c}	26^{a}	21^{d}
Overall mean	**0.40**	**0.74**	**0.88**	**0.41**	**17.7**	**13.0**	**1.4**	**20.2**	**25.5**

Where: BTH = breadth thickness, CTH = cotyledon thickness, LTH = seed length, ABA = Abscisic acid, MC = moisture content, figures followed by the same superscript letters are statistically similar.

Table 2. Physico-chemical composition of the six accessions of *G. latifolia* in 2009

Accessions	Physical Characteristics				Chemical Characteristics				
	BTH (mm)	CTH (mm)	LTH (mm)	100-seed weight (g)	ABA (%)	FAT (%)	Phenol (mg/100g)	Protein (%)	MC (%)
AC-01	0.39^{a}	0.72^{b}	0.87^{c}	0.39^{c}	19^{a}	14^{b}	0.9^{b}	19^{c}	20.0^{d}
AC-02	0.39^{a}	0.82^{a}	0.85^{c}	0.40^{c}	18^{a}	17^{a}	0.8^{b}	20^{b}	19.8^{d}
AC-03	0.39^{a}	0.59^{c}	0.85^{c}	0.31^{e}	20^{a}	8^{c}	2.0^{a}	21^{b}	25.0^{c}
AC-04	0.40^{a}	0.83^{a}	0.97^{a}	0.46^{b}	13^{c}	18^{a}	2.2^{a}	19^{c}	29.2^{a}
AC-05	0.41^{a}	0.87^{a}	0.92^{b}	0.54^{a}	9^{d}	13^{b}	1.8^{a}	22^{b}	26.4^{b}
AC-06	0.37^{a}	0.60^{c}	0.79^{d}	0.35^{d}	17^{b}	17^{a}	1.0^{b}	28^{a}	18.8^{e}
Overall mean	**0.39**	**0.72**	**0.88**	**0.41**	**16.0**	**14.5**	**1.5**	**21.5**	**23.2**

Where: BTH = breadth thickness, CTH = cotyledon thickness, LTH = seed length, ABA = Abscisic acid, MC = moisture content, figures followed by the same superscript letters are statistically similar.

Table 3. Seedling emergence capacity of the accessions of *G. latifolia* in both years

Accessions	2008				2009			
	CDP	CVE	MDG	CEP (%)	CDP	CVE	MDG	CEP (%)
AC-01	6^{a}	9.7^{b}	5.9^{d}	57^{e}	6^{a}	9.8^{b}	6.2^{c}	63^{c}
AC-02	7^{a}	8.0^{b}	12.0^{b}	74^{c}	6^{a}	8.4^{c}	11.7^{b}	80^{b}
AC-03	5^{b}	11.7^{a}	8.6^{c}	55^{e}	4^{b}	11.8^{a}	9.2^{b}	62^{d}
AC-04	5^{b}	11.7^{a}	17.9^{a}	90^{b}	4^{b}	11.9^{a}	20.1^{a}	94^{a}
AC-05	5^{b}	11.7^{a}	20.0^{a}	98^{a}	3^{b}	12.0^{a}	22.2^{a}	98^{a}
AC-06	5^{b}	11.5^{a}	11.0^{b}	61^{d}	4^{b}	11.6^{a}	11.3^{b}	69^{c}
Overall mean	**5.5**	**10.7**	**12.6**	**72.5**	**4.5**	**10.9**	**13.5**	**77.7b**

Where: CDP = cumulative dormancy period, CVE = coefficient velocity of emergence, MDG = mean daily germination, CEP = cumulative emergence percentage, figures followed by the same superscript letters are statistically similar.

Table 4. Correlation coefficient of the physio-chemical traits of the *G. latifolia* accessions

	ABA	BTH	CDP	CEP	CTH	CVE	FAT	LTH	MC	MDG	PHE	PRO	SWT
ABA	_												
BTH	-0.21	_											
CDP	0.29	-0.09	_										
CEP	-0.76**	0.09	-0.06	_									
CTH	-0.57*	-0.05	-0.01	0.73**	_								
CVE	-0.28	-0.02	-0.52*	0.17	-0.23	_							
FAT	-0.22	0.01	0.11	0.35	0.31	-0.09	_						
LTH	-0.39	0.21	-0.12	0.55*	0.51*	-0.11	0.34	_					
MC	-0.80**	0.17	-0.25	0.927**	0.66*	0.30	0.24	0.57*	_				
MDG	-0.69*	0.05	-0.18	0.94**	0.62*	0.35	0.29	0.41	0.87**	_			
PHE	-0.36	0.12	-0.43	0.53*	0.21	0.57*	-0.23	0.39	0.64*	0.59*	_		
PRO	-0.10	-0.07	-0.38	-0.28	-0.36	0.38	-0.07	-0.41	-0.30	0.11	-0.19	_	
SWT	-0.28	0.93**	-0.10	0.93**	0.93**	-0.24	0.35	0.50*	0.70**	0.91**	0.36	-0.37	-

** = significance at 1 %, * = significant at 5 %

Where: ABA = Abscisic acid, BTH = breadth thickness, CDP = cumulative dormancy period, CEP = cumulative emergence percentage, CTH = cotyledon thickness, CVE = coefficient velocity of emergence, LTH = Seed length, MC = moisture content, MDG = mean daily germination, PHE=Phenol content, PRO= Protein and SWT=Seed weight.

Table 5. Variance estimates of the physico-chemical traits of the accessions in both years

	2008						2009					
Traits	**σ^2e**	**σ^2g**	**σ^2p**	**GCV (%)**	**PCV (%)**	**H^2bs (%)**	**σ^2e**	**σ^2g**	**σ^2p**	**GCV (%)**	**PCV (%)**	**H^2bs (%)**
Absisic acid	4.42	14.48	18.90	21.37	24.39	76.61	5.6	13.85	19.45	23.25	27.56	71.21
Fat	4.25	16.35	20.61	31.08	34.92	79.37	4.9	15.46	20.36	27.12	31.12	75.93
Moisture content	0.21	13.90	14.11	1.26	1.27	98.51	0.52	10.60	11.12	15.20	15.57	95.32
Phenol	0.03	1.26	1.29	91.11	93.28	97.67	0.06	1.25	1.31	77.11	78.93	95.42
Protein	3.33	9.10	12.43	14.96	17.48	73.21	3.81	8.89	12.7	13.86	15.58	70.00
Cumulative Emergency Percentage	16.25	324.25	340.50	24.84	25.45	95.23	18.40	363.25	381.65	24.38	24.99	95.18
Seed breadth	0.32	0.69	1.01	2.10	2.25	68.37	0.33	0.55	0.88	1.90	2.26	62.50
Cotyledon thickness	0.0074	0.0133	0.0207	1.8	2.81	64.25	0.0066	0.012	0.0186	14.82	18.45	64.52
100-Seed weight	0.0001	0.0053	0.0054	20.57	20.64	98.1	0.0001	0.007	0.0071	17.83	17.99	98.6

Where: σ^2e = environmental variance, σ^2g = genetic variance, σ^2_p = phenotypic variance, GCV = genotypic coefficient of variance, σ^2_{PCV} = phenotypic coefficient of variance and H^2_{bs} = broad sense heritability

DISCUSSION

The significant differences in the physico-chemical traits of seeds of different accessions resulted in variation in seed emergence rates and seedling vigour. This is in agreement with an earlier report that larger seeds with larger embryos that differentiate crops into genetic lines is associated with increased germination (Lopez-Castaneda *et al*. 1996). Further, different seed sizes have been shown to influence water potential of winter wheat (*Hordeum vulgare*) seeds and their speed of germination as well as uniformity of germination and subsequent seedling development (Mian and Nafziger, 1994; Seiwa and Kenji, 2000). From the foregoing, the combined effect of the physical and chemical traits of the different genetic lines determined their qualities which resulted in varying emergence rates and seedling vigour.

The accession, AC–05 had larger seeds as well as higher seed breadth and cotyledon thickness than other studied accessions in both years. The consistency in size of the seeds of the accession over others for the two years indicates stability in the size of the seeds of the accessions. Larger seeds in maize are a physical trait that has been linked to contribute to higher germination and vigour when compared to smaller seeds (Batistella *et al.*, 2002). The size of *G. latifolia* seeds seems to influence germination in the species as AC-03 and AC–06 with the least cotyledon thickness and seed size gave significantly ($p < 0.05$) lower emergence rate whereas the accession AC–05 with larger seeds gave higher emergence rates.

The second year planting that gave higher emergence rate in all the accessions could be attributed to reduced moisture content in the seeds as the seeds had longer drying period of two weeks. Moisture content of seeds has been shown to have a marked influence on the life and vigour of the seed and emerging seedlings, respectively (ISTA, 2008). Seed germination of the crop species have been shown to increase with reduction in moisture after picking of follicles (Agbo and Obi, 2008). The significantly varying levels of the chemical components of the seeds of the accessions showed remarkable differences in the emergence rates of the species. For example, accessions AC–01 and AC–03 with high levels of ABA had lower emergence while AC–05 and AC-04 with lower levels of ABA had significantly higher emergence rates. The reports of Yogeesha *et al.* (2006) on the influence of ABA on dormancy mechanism in egg plant species corroborates with the results of this research. Higher level of ABA in matured seeds of some of the accessions is contrary to the report of Black (1991) that ABA is generally low or even undetectable in mature seeds.

Phenol that had been reported to induce dormancy in some crop species (Batistella *et al.*, 2002) was found to enhance emergence in *G. latifolia* species. This is indicated in the positive significant correlation ($r = 0.53$; $n = 36$) between phenol and cumulative emergence percentage in the accessions. The negative and significant relationship between ABA and emergence capability of the accessions further illustrates the influence of ABA in increasing dormancy when it is at higher levels in the species. Further drying of the species also reduces the level of ABA as seen in the level in the seeds in the second year with reduced moisture content. Similar results have been reported by Yogeesha *et al.* (2006) in *Solanum melongena* L.

Higher level of phenotypic variance and phenotypic coefficient of variation than the corresponding genetic components of the seed physico-chemical traits indicate interaction of the environment on the traits (Allard, 1960; Uguru, 1995). The highest level of genotypic and phenotypic variance among the accessions on the measured traits occurred on the phenol content that was observed to promote emergence in the species. This gives room for selection of accessions based on the trait. Heritability estimates were higher in the chemical than the physical traits of the seeds with the exception of seed weight. This indicates higher environmental influence on the physical traits and is suggestive that the chemical traits are more heritable and promising for selection.

Conclusion: The study has shown that there exist significant differences in the physico-chemical seed traits of the *G. latifolia* accessions. The genetic variation in the traits resulted in varying seedling emergence rate and vigour. AC-05 had larger seeds (0.53g) as well as higher seed breadth and cotyledon thickness but lower level of ABA in both years. The seedling emergence capacity of the accessions in both years showed that the seedlings emerged earlier and more vigorous in the second year with reduced moisture content. The second year also had reduced ABA. Accessions with larger seeds as well as lower ABA had higher and more vigorous emergence suggesting the impact of genetic factors in determining the performance of accessions. There was higher environmental influence on the physical traits of the species than the chemical traits resulting in higher heritability estimates for the chemical traits. Furthermore, the correlation coefficients suggested that accessions with larger seeds also have lower ABA and significantly higher vigorous emergence. It was concluded that different accessions showed differences in their seed characteristics which directly influenced emergence rate and vigour of seedlings over the two years.

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