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EFFECT OF PRE-SOWING HARDENING TREATMENTS USING VARIOUS PLANT GROWTH SUBSTANCES ON COWPEA GERMINATION AND SEEDLING ESTABLISHMENT

Audi, A. H* and Mukhtar, F. B.
Department of Biological Sciences, Faculty of Science, Bayero University, PMB 3011, Kano
Correspondence Author: audigenesis@daad-alumni.de

ABSTRACT
Seed germination and seedling establishment of two cowpea varieties IT87D-941-1 (IPI) and Kanannado (LPI) were carried out in the laboratory in Kano, Nigeria under the prevailing laboratory conditions of 25±2°C and 45-60% relative humidity. The cowpea seeds were pre-soaked in various solutions of 5ppm and 10ppm Indole acetic acid (IAA), Gibberellic acid (GA_3) and Ascorbic acid (AA), concentrations respectively. The soaked seeds were air dried for 24hrs and thereafter sown in various flasks containing dried cotton wool. The result of the study showed significant difference in percentage germination among the cowpea varieties and hormone concentrations (P<0.001). Seeds treated with 5ppm IAA and GA_3 showed significant increases in percentage germination and seedling growth in the two cowpea varieties. Germination and seedling growth decreased markedly with increasing hormone concentration. Seeds pre-soaked in distilled water responded poorly. Based on these results, 5ppm concentrations of IAA and GA_3 were found to be the best for enhancing seedling growth in cowpea and it is therefore recommended for cowpea seedling establishment. The result also emphasized that pre-sowing hardening treatment of cowpea seeds in IAA and GA_3 could significantly enhance their germination and seedling growth. This suggested that hormone treated cowpea seed have the potential of overcoming adverse effect of water stress in tropical Savannah.

Keywords: Plant growth substances, Cowpea, Germination, Seedling Establishment

INTRODUCTION

Cowpea (*Vigna unguiculata* (L) Walp) (Leguminosae: Papilionoidae) represent the main food legume and a versatile crop in tropical Africa. It is drought tolerant and could produce better growth in warm climates. It is most popular in the semi arid regions of the tropics where other food legumes are available (Singh and Sharma, 1996). The crop has been described as the major source of dietary protein in tropical and subtropical regions of the world especially where animal protein consumptions are low (Opareke *et al*., 1998). Efforts made to maximize yield, is largely hampered by adverse effect of a biotic stress such as salinity and drought. These effects cause a huge loss due to law yield and failure of the crop to establish in some cases. Alternative approach towards efficient and cost effective means of production of cowpea in the tropical Savannah is very desirable. Pre-sowing hardening seed treatment is an easy, low cost and low risk technique and also an alternative approach recently used to overcome the effect of abiotic stresses in agricultural production. It is found to be efficient in improving seed emergence and growth of crops (Bradford, 1986: Parera and Cantiliffe, 1991). Pre-sowing hardening treatment is a repeated soaking and control seed hydration in solution containing organic or inorganic solute (Heydecker *et al.,* 1973; *Khan et al.,* 1980; Dane berger *et al.,* 1992; Pill and Necker, 2001), followed by redrying that allows pregerminative metabolic activities but prevent radicle emergence (Young *et al.,* 1977; Bradford, 1986; Khan, 1992; Pill, 1994 and Basra *et al.,* 2003). The hardening treatment proved to be better for vigour enhancement than the traditional soaking (Basra, *et al.,* 2005). It was reported clearly that the hardening treatment enhance seeds vigour by protecting structure of the plasma membrane against injury during stress (Bewley and Black, 1982; JunMin *et al*., 2000). It is a well establish fact that, pre-soaking seeds with optimal concentration of phytohormones enhance their germination, growth and yield of some crop species under condition of environmental stress by increasing nutrient reserves through increased physiological activities and root proliferation (Asana *et al*., 1955; Dave and Gaur, 1970; Garg and Srivastava, 1970; Singh and Darra, 1971; Darra *et al*., 1973; Bozeuk, 1981; Risvi, 1994).

Previous studies have also shown that presowing seed treatment in various concentration of Indole acetic acid (Ozturk et al., 1993; Zaidi and Singh, 1993; Hegazi *et al.,* 1995; Kumar and Singh 1996; Gulnaz *et al.,* 1999), Gibberellic acid (Ungar, 1977; Warieng, 1982; Cohn and Castle, 1984; khan and Risvi, 1994; Keely and Fotherringham, 1997; Mella *et al*., 1997; Radi *et al.,* 2001; Anguish *et al.,* 2001) and Ascorbic acid (Verma and Srivastava, 1998; Roy and Srivastava, 1999; Roy and Srivastava, 2001; Alhakimi and Hamada, 2001) may promote or inhibit seedling growth. However little is emphasized on how plant growth hormones could affect cowpea seed germination and seedlings growth. The main objective was to assess the physiological effect of $Indole_3$acetic acid (IAA), Gibberallic acid (GA_3) and Ascorbic acid (AA) on germination and seedling growth of cowpea.

MATERIALS AND METHODS

The study was carried out in the Physiology Laboratory, Department of Biological Sciences Bayero University Kano, Nigeria. Cowpea seeds used for the experiments were obtained from the International Institute of Agriculture (IITA), Kano Research Station. The cowpea accessions were IT87D-941-1 which is an improved, photo-insensitive variety (IPI). Brown in colour, rough in texture and Kanannado variety, which is local photo-sensitive (LPI), white in colour and also rough in texture. These seeds were sterilized after weighing using 75% ethanol in 250ml conical flask.

Pre-Sowing Hardening Treatments

Different concentrations of the growth substances prepared in the laboratory were transferred from the reagent bottles into 50mls conical flasks which were clearly labelled according to the concentration of the growth substances to be used in the soaking treatment. Some quantities 5ppm and 10ppm each of Indole acetic acid (IAA), Gibberellic acid (GA_3) and Ascorbic acid (AA) were used for soaking the seeds separately. There were actually two controls; distilled water treatment and the untreated seeds which were not presoaked before sowing. The seeds were soaked in the various concentrations of the growth substances and the distilled water for a period of an hour after which they were drained using cotton wool and allowed to dry on filter papers for 24hrs before sowing. The weight of each treated seed variety was taken after drying using a weighing balance. Distilled water was also used for soaking and to also serve as control (Darra *et al.,* 1973) so that the effect of seed pretreatment on plant growth should not be affected by the differences in seed development along with untreated seeds for comparing the effect of various pre-treatments.

Planting of Seeds

After drying, the seeds were sown on moist filter papers in 9cm well labelled Petri dishes. Into each petridish, 10 seeds were sown for each hormone concentration and this was replicated 3-times making a total of 48-samples (Petri dishes). The Petri dishes were covered and left by the window side of the laboratory. The seeds were inspected at interval and moistened regularly with water. Each replicate was weighed after 2hours, 4hours, 6hours and every 24hours by means of a digital weighing balance to note the weight change. Observation was made daily for 7-days period during which any seeds germinated were recorded. This was maintained for a period of 3weeks after which growth of seedlings (shoot length and root length) was measured using a thread and meter rule. These procedures were repeated three times at intervals to facilitate computation of the recorded data. The experiment was laid out in a completely randomize design. All percentages were transformed prior to analysis. Data obtained were subjected to two ways ANOVA in randomized block design using the Genstat Statistical Software (1995 version). Rate of imbibition was taken as change in fresh weight.

RESULTS

Seed germination varied significantly among the varieties and hormone concentrations ($P<0.001$). Percentage germination in the two cowpea varieties increased with increase in number of days after sowing (Table 1). The results showed significant increase in the germination percentage for seeds presoaked in the various hormones when compared with distilled water and the unsoaked seeds that had no treatment. Maximum increase of up to about 90% and 73% for IT87D-941-1 and Kanannado presoaked in 5ppm IAA was observed. This was followed by 5ppm each GA_3 and AA treatment in Kanannado (83 and 77%) and IT87D-941-1 (67% respectively. Lower percentage germination was observed in distilled water treated seeds (50 and 33%) for IT87D-941-1 and Kanannado respectively, and the least was recorded in the unsoaked seeds (Table 1). Comparison of the treatments shows that 10ppm GA_3 and AA resulted in significantly ($P<0.001$) lower percentage germination in IT87D-941-1 (66.6% and 63.3%) respectively and in Kanannado (53.3%).

Tables 2 and 3, shows the rates of imbibition in the two cowpea varieties. There was progressive increase in weight as the number of hours increased from 2 to 168hrs in both the treated seeds and the controls. The rate of imbibition varied significantly ($P<0.001$) in the two cowpea varieties as a result of the hormone treatments. Greater imbibition was recorded in the hormone treated seeds of the two varieties by 168hrs after sowing compared with the distilled water treated and the untreated seeds (Tables 2 and 3). Maximum weight change was however observed in seeds presoaked in 5ppm IAA followed by 5ppm GA_3 and AA in IT87D-941-1(9.0, 8.4 and 8.0g) and Kanannado (12.4, 10.5 and 10.0g) respectively. Least weight change was observed in the controls in both cowpea varieties. Water uptake was initially rapid at 2hrs of imbibition then it slowed down with a short lag phase between 2-6hrs but picks up again at accelerating phase from 6-72hrs in IT87D-941-1. In Kanannado, the lag phase lasted from 6-24hrs while the second phase of rapid growth was from 24-72hrs (Tables 2and 3).

Root and shoot lengths also varied with different hormone concentration ($P<0.001$) as presented in Table 4. Significant increase in lengths of root and shoot were observed in the hormone treated seeds when compared with the controls (distilled water and untreated seeds) the exception was the root lengths of Kanannado pre-soaked in 10ppmAA(3.8cm) which did not differ significantly from distilled water treated seeds(3.7cm). Root lengths were markedly higher in seeds presoaked in lower hormone concentration (5ppm GA_3 and 5ppm IAA respectively). The variety, IT87D-941-1 had great root lengths (8.9 and 8.0cm) than Kanannado (6.8 and 5.7cm). These were induced by 5ppm GA_3, 5ppm IAA (Table. 4). Conversely, the shoot length was greater in the seeds presoaked in higher concentrations, which are 10ppm IAA and 10ppm GA_3. The longest shoot was recorded in IT87D-941-1 seeds germinated following 10ppm IAA and GA_3 pre-sowing treatments with 14.8 and 16.2cm respectively.

Table 1. Percentage Germination for the 2-Cowpea Varieties (IT87D-941-1 and Kanannado) presoaked in various Concentrations of IAA, GA_3 and AA

	Percentage germination/day (%)							
	IT87D-941-1				Kanannado			
Hormone hardening treatment	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
5ppm IAA	33.3	56.6	80.0	90.0	20.0	40.0	63.3	73.3
10ppm IAA	26.6	46.6	66.6	76.6	16.6	30.0	50.0	56.6
5ppm GA_3	36.6	50.0	70.0	83.3	16.6	36.6	60.0	66.6
10ppm GA_3	23.3	43.3	63.3	66.6	13.3	26.6	50.0	53.3
5ppm AA	26.6	46.6	56.6	76.6	16.6	23.3	53.3	66.6
10ppm AA	23.3	40.0	50.0	63.3	13.3	16.6	43.3	53.3
Dist H_2O	16.6	30.0	43.3	54.0	10.0	13.3	33.3	43.3
Untreated	10.0	16.6	30.0	46.6	6.6	16.6	30.0	36.0
Mean X	24.54	41.21	57.47	69.62	14.12	25.37	47.90	56.12
LSD (5%)	3.918	5.777	8.237	6.953	2.015	3.837	4.339	4.546

Data are means of 3-determinations (n=3).

Table 2. Rate of Imbibition in Germinating Cowpea Seed (Kanannado Variety) as affected by different Hormone Concentrations of IAA, GA_3 and AA

	Weight change/hrs										
Hormone hardening treatment	**0hrs**	**2hr**	**4**	**6**	**24**	**48**	**72**	**96**	**120**	**144**	**168**
5ppm IAA	2.0	4.5	4.8	5.2	5.6	9.2	11.0	12.0	12.2	12.4	12.4
10ppm IAA	2.0	4.6	4.9	5.4	6.4	8.8	10.0	10.4	10.5	10.5	10.6
5ppm GA_3	2.0	4.7	4.9	5.2	6.0	8.2	9.3	9.3	10.1	10.3	10.5
10ppm GA_3	2.0	4.5	4.7	5.0	6.0	8.2	9.1	9.1	9.7	9.8	9.8
5ppmAA	2.0	4.6	4.9	5.2	6.0	8.2	9.3	9.3	9.9	9.9	10.0
10ppm AA	2.0	4.0	4.3	4.6	5.6	7.4	8.3	8.3	8.8	8.9	9.2
Dist H_2O	2.0	4.4	4.6	4.9	5.2	6.2	7.4	7.4	8.2	8.2	8.3
Untreated	2.0	4.4	4.5	4.7	5.3	6.0	6.3	6.3	6.5	6.5	6.8
Mean	2.0	4.46	4.70	5.02	5.76	7.77	8.83	9.01	9.48	9.56	9.70
LSD (5%)	NS	NS	NS	NS	0.112	0.900	0.402	0.477	0.480	0.453	0.454

Data are means of 3-determinations (n=3).

Table 3. Rate of Imbibition in Germinating Cowpea Seed (IT87D-941-1 Variety) as affected by different Hormone Concentrations of IAA, GA_3 and AA

	Weight change/hrs										
Hormone hardening treatment	**0hrs**	**2hr**	**4**	**6**	**24**	**48**	**72**	**96**	**120**	**144**	**168**
5ppm IAA	2.0	4.5	4.6	4.8	5.8	7.2	8.2	8.2	8.7	8.9	9.0
10ppm IAA	2.0	3.9	4.0	4.1	5.0	6.0	6.9	7.3	7.5	7.5	7.5
5ppm GA_3	2.0	3.9	4.2	4.3	5.0	6.5	7.6	8.1	8.2	8.2	8.4
10ppm GA_3	2.0	3.5	3.7	3.8	4.4	5.8	6.8	7.3	7.4	7.4	7.5
5ppmAA	2.0	3.8	3.9	4.0	5.0	6.2	7.0	7.6	7.8	7.8	8.0
10ppm AA	2.0	3.4	3.5	3.7	4.8	5.6	6.8	7.0	7.0	7.0	7.1
Dist H_2O	2.0	3.0	3.2	3.4	4.6	5.4	6.2	6.6	6.6	6.7	7.0
Untreated	2.0	2.6	3.0	3.2	4.4	5.2	6.0	6.4	6.6	6.7	6.8
Mean	2.0	3.57	3.76	3.91	4.87	5.98	6.93	7.31	7.47	7.52	7.66
LSD (5%)	NS	0.160	0.143	0.138	0.123	0.76	0.192	0.176	0.203	0.207	0.206

Data are means of 3-determinations (n=3).

Table 4. Mean Length of Root and Shoot (cm) of the Cowpea Varieties IT87D- 941-1 and Kanannado Presoaked in Various Hormone Concentrations taken at 7- Days after Sowing

	Root length		Shoot length	
Hormone hardening treatment	**IT87D-941-1**	**Kanannado**	**IT87D-941-1**	**Kanannado**
5ppm IAA	8.0	6.8	6.2	11.2
10ppm IAA	6.6	4.7	8.0	14.8
5ppm GA_3	8.9	5.7	7.2	13.8
10ppm GA_3	7.8	5.5	8.5	16.2
5ppmAA	6.5	5.4	6.2	11.0
10ppm AA	5.3	3.8	6.5	12.5
Dist H_2O	3.5	3.7	5.2	9.2
Untreated	2.0	2.4	3.5	7.4
Mean	6.07	4.75	6.41	12.01
LSD (5%)	1.926	1.138	1.293	2.390

Data are means of 3-determinations (n=3).

DISCUSSION

Seeds showed variable responses to presowing hardening treatments with respect to germination. Soaking in 5ppm IAA GA_3 and AA produced higher germination percentages than seeds soaked in 10ppm hormone concentrations. Pretreatment in 5pmm IAA gave maximum seed germination on the 4th day (96hrs of imbibition) with about 90%and 73% for IT87D-941-1 and Kanannado varieties respectively. This conforms with the work of Ventura (1987) who reported that IAA stimulates germination of *Lupinus albus* at concentration of less than 10mMl.rate of germination in terms of radicle emergence was faster in IT87D-941-1 compared with Kanannado (Table 1). The delayed radicle emergence observed in Kanannado could be attributed to differences in the composition of seed cotyledons and hardness of seed testa (Ashram and Iram, 2002; Mukhtar and Alhassan, 2006). Seeds presoaked in distilled water had higher percentage germination than the unsoaked seeds. This pattern agrees with the reports of Rehman *et al.* (1998) and Kamboh *et al.* (2000) that presoaking treatment in distilled water exhibit considerable effectiveness on germination and later growth in different plant species under saline and non-saline conditions. Another observation also reported the ineffectiveness of presoaking in distilled water to improve germination and growth in wheat (Chaudhuri and Weibe, 1968) and Kentucky blue grass (*Poa pratensis*) (Pill and Necker, 2001).

Rate of imbibition in terms of change in fresh weight as presented in tables 2 and 3 showed that the pre-sowing soaking treatments increased the rate of imbibition particularly in the seeds presoaked in lower concentration (5ppm) of IAA, GA_3 and AA by 168hrs. This result can be related with the findings of Northern (1972); Salisbury and Ross (1997) that hormones generally decrease viscosity of cytoplasm and increase diffusion of water into the cell. Not only decreasing the viscosity of the cytoplasm, the hormones may induce growth by production of substances within the endosperm prior to radicle emergence, which may as well increase the osmotic potential of the cell. This observation could be related with the work of Dias *et al.* (1993) who reported that growth of embryo increases the osmotic potential thereby increasing water uptake into the cell. Water uptake was initially rapid up to 2hrs of imbibition prior to radicle emergence but slowed down with a short and steady lag phase as it picked up again at accelerating rate subsequent to radicle emergence from 6 and 24hrs in IT87D-941-1 and Kanannado varieties respectively. The initial rapid uptake of water may be due to the low level of moisture content in the seeds and as water enters the cells, the osmotic potential of the cell is raised and this causes water to enter until the cells become fully turgid (Taiz and Zeiger, 2002). The slow water absorption after the first 2hrs may be associated with the growth of radicle within the seed coat and as the radicle emerged water was absorbed at an accelerating rate thus marking the second phase of rapid water uptake (Taiz and Zeiger, 2002). In Kanannado variety, the lag phase was observed to be longer from 2hrs up to about 24hrs of imbibition before it picked up again. Hence it can be deduced that rate of imbibition was faster in IT87D-941-1 indicating that, the seed reached saturation stage and became turgid earlier than the Kanannado variety, prior to radicle emergence (Tables, 2and 3). Generally growth response of the two cowpea varieties (IT87D-941-1 and Kanannado) with respect to the presowing-hardening treatments in various growth hormones show variable growth pattern at different stages of their development (germination, shoot and root growth). Root lengths in the two varieties increased with decrease in the hormone levels whereas shoot lengths increased as the hormone concentrations increased. This observation conforms with the findings of Singh (1996); Gulnaz *et al*. (1999); Khan *et al*. (2002) that, the quantitative and qualitative responses of plants to different hormones may differ considerably at different plant growth stage. The variety, IT87D-941-1 had the greatest root length than Kanannado. These were induced by 5ppm GA_3, 5ppm IAA while higher concentrations (10ppm) of the hormones enhance longer shoot growth in Kanannado (Table 4).

The production of high root in plant raised from seeds treated with these hormones suggests that the rate of absorption of available nutrients might have significantly been enhanced. Darra *et al*. (1977) suggested that plant hormones increase the rate of absorption of water and available nutrients thereby resulting in better growth. The hormones might also have substantially enhanced cell enlargement and rapid increase in cell division as suggested by (Magome, 2004). The development of cowpea presoaked in Ascorbic acid (AA) was observed to be consistently lower when compared with that of Indole acetic acid (IAA) and GA_3 treated cowpeas.

CONCLUSION

The findings of this study revealed that, cowpea germination and early seedling growth were promoted by pre-sowing hardening treatments in IAA and GA_3. The lower concentration of these hormones (5ppm) was found to be more effective in inducing germination imbibition and root development of the two cowpea varieties whereas 10ppm GA_3 stimulated greater shoot growth of the seedlings.

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