



EFFECT OF FOLIAR FERTILIZATION WITH LIQUID NITROGEN AND BENZYL AMINO PURINE ON SENESCENCE, NITROGEN MOBILIZATION AND CARBON CONTENT IN COWPEA VARIETIES

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ABSTRACT

The effects of liquid nitrogen fertilizer (150% N) and Benzyl Amino Purine (200ppm BAP) were investigated on senescence, nitrogen mobilization and carbon content in two cowpea varieties during the rainy and dry seasons. This study was conducted in the screen house at the University of Lagos located in the south western part of Nigeria. There were four treatments (nitrogen fertilizer, BAP, combined treatment of liquid nitrogen fertilizer and BAP and then the control). Data was collected on senescence, nitrogen and carbon content. The result on senescence showed that onset of senescence, days to 50% senescence, 90% senescence and days to total death of the plants occurred earlier in IT89KD – 288 than in Kanannado, while comparison between the treatments showed that senescence started earlier in plant treated with single treatment of liquid nitrogen fertilizer (15%N). Between the two seasons, senescence started earlier in the dry season than in the rainy season. Result on Nitrogen showed that between the two varieties, IT89KD – 288 had greater Nitrogen content than Kanannado at 6 and 9 weeks, but at week 12 there was a decrease of the nitrogen in the leaves of IT89KD-288 then in Kanannado. The treatment with the highest Nitrogen content in the varieties was combined treatment of 150% N fertilizer + 200ppm BAP at 6, 9 and 12 weeks, followed by Nitrogen fertilizer (150% N) at 6 weeks only, but at 9 and 12 weeks when subsequent analysis was carried out there was more Nitrogen in the leaves treated with 200ppm BAP. There was more Nitrogen in the dry season than in the rainy season. With regards to carbon content Comparison of the two varieties has shown that there was more carbon in IT89KD-288 than in kanannado at 6 and 9 weeks but the difference was not significant, but at 12 weeks when senescence had started in IT89KD-288 there was more carbon in the leaves of kanannado and the difference between them was significant. Between the treatments, combined treatment of nitrogen fertilizer and BAP (150%N +200ppm BAP) had the highest carbon content at 6, 9 and 12 weeks. The implication of this study is that the treatments delayed senescence in the cowpea varieties and slowed down nitrogen mobilization and increased carbon content. These were more pronounced in the local variety kanannado and the single treatment of 200ppmBAP and therefore recommended for use by the farmers to delay senescence.

Keywords: Cowpea, Benzyl Amino Purine, Nitrogen, Kanannado, Fertilizer.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is an important leguminous crop throughout sub-Saharan Africa, as well as in some parts of the Americas and Asia. It contributes a very important and inexpensive source of protein, providing more than half of the plant protein in human diets. It contains about 23 – 25% of protein (Singh, 1997). Cowpeas are grown in a wide range of environment from 40°N to 30°S and in lowland and highland ecologies. They are principally grown in West Africa, but are also grown in Asia, Latin America and North America (Rachie, 1985).

Senescence means aging that ultimately leads to death. Plant aging and environmental stresses may induce the process of senescence. In plants senescence is a complex and highly regulated process that occurs as part of plant development. It is a pre-planned process, which can be seen in annuals such as rain crops that turn from green to golden as the grain ripens – before

harvest. These changes visible to the naked eye are accompanied with internal biochemical events; in which nutrients are being transferred from the leaves to other organs in the plant to be used in its development (Green and Jones, 1982).

Nitrogen is one of the most expensive nutrients to supply and commercial fertilizers represent the major cost in plant production. Plants have a fundamental dependence on inorganic nitrogen and 85–90 million metric tonnes of nitrogenous fertilizers are added to the soil worldwide annually (Good *et al.*, 2004). Leaf proteins and in particular photosynthetic proteins of plastids are extensively degraded during senescence, providing an enormous source of nitrogen that plants can tap to supplement the nutrition of growing organs such as new leaves and seeds (Lim *et al.*, 2007). Leaf senescence is not only essential for nitrogen mobilization, but evidence has shown that leaf senescence is also important for yield delaying leaf senescence results in a prolongation of photosynthesis that increases grain

yield and carbon filling into seeds. Breeding plants have to cope with the dilemma that delayed senescence could lead to higher yields but also to a decrease in NRE and to a decrease in grain protein content. On the other hand, increasing nitrogen remobilization has the advantage of re-using nitrogen from the vegetative parts and of lowering nitrogen loss in the dry remains (Keech *et al.*, 2007).

In cowpea senescence causes substantial reduction in total grain yield because most cowpea plants die after producing the first flush of pods. The reduction in yield is most drastic in the local varieties (Weaver and Amasino, 2001). Delaying leaf senescence will most probably extend the reproductive period and increase the photosynthetic efficiency of the crop resulting in increased grain yield. The objective of the experiment is to investigate the combined effect of liquid nitrogen fertilizer with cytokinin (BAP) in regulating the onset of senescence in some cowpea varieties and also to determine the combined effect of nitrogen fertilizer and BAP on yield in cowpea. The aim of the research is to evaluate the combined effect of nitrogen fertilizer and BAP on senescence, nitrogen mobilization and carbon content in cowpea varieties and the objectives include: To investigate the combined effect of liquid nitrogen fertilizer with cytokinin (BAP) in regulating the onset of senescence in some cowpea varieties, to evaluate the effect of the combined treatments on nitrogen mobilization and to determine the combined effect of nitrogen fertilizer and BAP on carbon content in cowpea.

MATERIALS AND METHOD

Study area

This study was conducted in the screen house at the University of Lagos located in the south western part of Nigeria. Lagos is situated at latitude 6°27'11"N, longitude 3°23'45" E and altitude 0-41m (0-135 ft). In the koppen climate classification, Lagos has a tropical wet and dry climate that borders on a tropical monsoon climate. Lagos experiences two rainy seasons, with the heaviest rains falling from April to July and a weaker rainy season in October and November. There is a brief relatively dry spell in August and September and a longer dry season from December to March. Monthly rainfall between May and July averages over 400 mm (16 in), while in August and September it is down to 200 mm (7.9 in) and in December as low as 25 mm (0.98 in). The main dry season is accompanied by harmattan winds from the Sahara Desert, which between December and early February can be quite strong. The highest maximum temperature ever recorded in Lagos was 37.3 °C (99.1 °F) and the minimum 13.9 °C (57.0 °F). (World weather information service Lagos, 2012)

PLANTING

Plastic pots of 250mm diameter were used in this study. They were filled with fresh sandy loam top soil and watered well for two days before planting. The treatments were replicated 8 times

using completely randomize design. The treatments used include Benzyl Amino Purine (BAP) and liquid Nitrogen fertilizer (Boost Extra). There were also combined treatments of BAP and liquid Nitrogen fertilizer. The first planting was done on the 9th of August, 2012 for the rainy season and the second planting was done on the 20th of January, 2013 for the dry season. Seeds were directly sown on the prepared pots (after surface treatment with fungicide Apron plus) at 2 seeds per pot. Later they were labeled and placed in completely randomized block design and labeled appropriately using white and green plastic tags according to the plant treatment applied.

DATA COLLECTION

Data was collected on senescence, the Changes in colour of leaves were observed visually and the extent of chlorophyll loss was monitored. Days to onset of senescence were counted and recorded for each plant, likewise days to 50% of senescence, days to 90% of senescence and days to complete death of the plant. From the data obtained the duration of senescence in the different treated and control plants were estimated in order to determine their effects on senescence in the cowpea varieties. Nitrogen and protein contents of the leaves at flower bud initiation stage and grains at harvest of the two cowpea varieties were determined. The analysis was carried out at Biochemistry laboratory of the University of Lagos using micro Kjeldahl method. One (1 gram) each of fresh leaves and the grains of each of the treated and control cowpea genotypes were supplied and their nitrogen content estimated. The estimated values were multiplied by 6.25 to get the percentage protein content. The procedure is as follows:

Determination of carbon

One gram (1.0g) of the leaves was a shed and cooled, the ash was dissolved in 10cm³ of 10% nitric acid and was made up to the mark in a 100cm³ volumetric flask with water. Carbon was determined at its respective wavelength using Alpha – 4 model of atomic absorption spectrophotometer (AAS).

Statistical Analysis

The data collected was subjected to analysis of variance and significant differences were further separated using least significance difference test at $P \leq 0.05$.

RESULTS

Senescence

The result of onset, progression and duration of senescence in the two cowpea varieties, is presented in Table 1. Comparison of the varieties showed that there was significant difference ($P \leq 0.05$) in all the parameters. In the variety Kanannado, there was a general delay in the number of days taken to the onset of

senescence and days to 50% senescence, days to 90% senescence, and days to total death of the plant, when compared with IT89KD – 288 (Table 1).

The result of the effect of the various treatments on senescence is also shown in Table 1. The treatments induced different responses in the number of days taken to the onset of senescence, days to 50% senescence, days to 90% senescence, and days to total death of the plants. Comparison between the treatments and the control showed that Nitrogen fertilizer hastened senescence, while BAP treatment applied as a single treatment or in a combination with N fertilizer delayed senescence.

The difference was significant ($P \leq 0.05$) (Table 1). Between all the treatments 150%N hastened the onset of senescence (66 days), and days to 50% senescence (82 days), 90% (96 days) and total death (105 days). There was delay induced by the combined treatment of 150% N +200ppm BAP in the onset of senescence (72 days), 50% senescence (96.00 days), 90% senescence (110) and total death of plant (121). Treatment with 200ppm BAP was observed to delay the onset of senescence (77 days), and 50% senescence (97 days), 90% senescence (106) and Total death of plant (120). There was no significant difference between 200ppm BAP treated plants and the combined treatment of 150% Nitrogen fertilizer + 200ppm BAP with respect to 90% senescence and total death of plants. Onset of senescence in the control plants occurred at 57 days after planting, 50% at 69 days, and 90% at 81 days and total death at 92 days after planting (Table 2). The duration of senescence from its onset to total death of plants ranged from 35 days in the control to 39 days in 150%N to 42 days in 200ppm BAP to 48 days in 150% N + 200ppm BAP (Table 2). This shows that reproductive period in the combined treatment 150% N + 200ppm BAP was most extended by about 13 days.

Effect of the two planting seasons (rainy and dry) on senescence is presented in Table 1. Onset of senescence, days to 50% and 90% senescence and total death of the plants were earlier in the dry season planting when compared with the rainy season planting with significant difference ($P \leq 0.05$). In the rainy season, senescence commenced by 69 days and total death of the plants occurred by 117 days after planting, whereas onset of senescence in the dry season was recorded at 67 days and total death at 102 days after planting (Table 1). The duration of senescence from onset to total death were 35 days in the dry season and 47 days in the rainy season (Table 1) suggesting that reproductive period was extended by about 12 days in the rainy season.

NITROGEN MOBILIZATION

Nitrogen content of the leaves

Table 2 shows the difference of the two varieties on Nitrogen contents of the leaves at different weeks after planting. Nitrogen content of the cowpea varieties decreased with increased in

number of weeks after planting with significant difference. The rate of decrease was observed to be faster in IT89KD-288 while it was more gradual in the local variety. Between the two varieties, IT89KD – 288 had greater Nitrogen content than Kanannado at 6 and 9 weeks, and the difference was significant, but at week 12 there was a decrease of the nitrogen in the leaves of IT89KD-288 and Kanannado had significantly higher percentage of the Nitrogen than IT89KD-288. The level of Nitrogen decreased in all the treatments and the control as the number of weeks after planting increased with significant difference ($P \leq 0.05$) (Table 2). The treatment with the highest Nitrogen content in the varieties was combined treatment of 150% N fertilizer + 200ppm BAP at 6, 9 and 12 weeks, followed by Nitrogen fertilizer (150% N) at 6 weeks only, but at 9 and 12 weeks when subsequent analysis was carried out there was more Nitrogen in the leaves treated with 200ppm BAP than with 150% N fertilizer and the difference between them was significant. The rate of decline in Nitrogen content was slower in 200ppm BAP treated plants followed by 150% N + 200ppm BAP while it was faster in the control and 150% N fertilizer treated plants. Likewise the rate of decrease was faster between 6 and 9 weeks after planting in all the treatments except 200ppm BAP (Table 2).

Regarding the two seasons in terms of Nitrogen, the result showed that N content decreased as the number of weeks after sowing increased from 6-12 weeks in both planting seasons and the difference was significant ($P \leq 0.05$). There was more Nitrogen in the dry season than in the rainy season and the difference was significant at 6 and 9 weeks but at 12 weeks dry season did not differ significantly from the rainy season (Table 2).

Nitrogen content of the grains at harvest

Table 3 shows the result of the nitrogen content of the grains. All the treatments resulted in greater nitrogen content compared with the control. Nitrogen content of the grains was higher in the variety IT89KD – 288 than in Kanannado and the difference between them was significant. ($P \leq 0.05$). The highest Nitrogen content was observed in the combined treatment of 150%N+200ppmBAP (4.355%), followed by the 150%N treatment (4.033%) then 200ppm BAP (3.724%), and then the control (3.405%) (Table 3).

Table 3 also shows the effects of the two planting seasons on N content of the grains. There was greater nitrogen content in the grains in the dry season than in the rainy season and the difference was significant ($P \leq 0.05$).

Carbon content in the leaves of the cowpea varieties.

There was significant decrease in the carbon content of the leaves of the cowpea varieties that is IT89KD-288 and Kanannado from 6 to 12 weeks after sowing. Comparison of the two varieties has shown that there was more carbon in IT89KD-

288 than in kanannado at 6 and 9 weeks but the difference was not significant, but at 12 weeks when senescence had started in IT89KD-288 there was more carbon in the leaves of kanannado and the difference between them was significant, also there was significant difference between all the weeks in both IT89KD-288 and kanannado (Table 4).

Carbon content in both the treated and control plants was observed to decrease with significant difference as the number of weeks after sowing increased from 6 to 12 weeks (Table 4). The result on the effect of the treatments on carbon content has shown that all the treatments differed significantly from the control. Between the treatments, combined treatment of nitrogen fertilizer and BAP (150%N +200ppm BAP) had the highest carbon content at 6, 9 and 12 weeks, it differed significantly from Nitrogen fertilizer (150%N) treated leaves at 9 and 12 weeks but at 6 weeks, there was no significant difference between the combined treatment and 200ppm BAP treated plants in all the weeks. Between Nitrogen fertilizer treatment (150%N) and 200ppm BAP, the results showed that there was more carbon with nitrogen fertilizer than with BAP at 6 weeks, but at 9 and 12 weeks there was more carbon in plants treated with BAP (Table 4).

Result on the effect of the two seasons has shown that there was a significant decline in the carbon content in both seasons from 6 to 12 weeks after sowing. Between the two seasons the result showed that there was more carbon in the dry season than in the rainy season at 6 and 9 weeks but the difference was not significant, but at 12 weeks there was more of it in the rainy season and the difference was significant (Table 4).

DISCUSSION

Senescence

Onset of senescence started in the variety IT89KD – 288 than in Kanannado likewise 50% senescence, 90% senescence and total death of the plant. Onset, progression and duration of senescence started earlier in those plants treated with 150% N this was followed by the combined treatment of 150% N +200ppm BAP. Nitrogen Fertilizer is not known to delay senescence. Several studies reported that N fertilizer is known to improve soil productivity and fertility which improved yield and quality of crops (Whalen, 2000; Maerere *et al.*, 2001; Vanek, 2003). Soil treated with N fertilizer was found to be loose. This probably provided adequate aeration in the soil and improved microbial activities (Xio *et al.*, 2006). On the other hand turmeric plant when treated with Nitrogen fertilizer remained green longer and resulted in a higher vegetative growth and yield (Mazid, 1993; and Seobi, 2005 and Anes and Johnson, 1980).

Single hormonal treatment i.e. 200ppm BAP and combined treatment 150% N significantly delayed the onset, progression and duration of senescence in both varieties and in both seasons.

Several workers such as Nooden *et al.*, (1979), TAO, (1983) and Richmond and Lang (1994), reported that BAP and gibberellins retard senescence while Abscissic acid and ethylene tend to act as accelerators. Leaf senescence can be retarded locally by the application of BAP (Smith and Watson, 1988). Physiological studies suggest that BAP can regulate leaf senescence and that the internal BAP level drops with the progression of leaf senescence (Smith and Watson, 1988). Senescence is the result of complex changes in basic plant metabolism. In higher plants, various degradative phenomena associated with free radicals (FRs) have been implicated in the senescence process (Leshem *et al.*, 1986 Thompson *et al.*, 1987a). In plants, senescence-associated parameters can effectively be retarded by cytokinins (BAP) and polyamines.

Nitrogen Mobilization

Increase in the nitrogen content was observed in all the plants given fertilizer and hormonal treatments. The variety IT89KD – 288 had greater nitrogen content in both the leaves and the grain than the variety Kanannado and the difference was significant. Between the treatments, greater nitrogen content of the leaves and the grains were recorded in the combined treatment of 150% N+ 200ppm BAP. It was reported that there is a close correlation between nitrogen content and Rubisco content during leaf senescence (Makino *et al.*, 1984). It was demonstrated that 'Akenohoshin' plant maintained greater amount of nitrogen in leaves during ripening, which might be responsible for the difference in the maintenance of high leaf levels of Rubisco between cultivars (Ookawa *et al.*, 2003).

The hormone BAP has effect on nitrogen and biomass partitioning in wheat seedlings (Simpson *et al.*, 1998) and a perennial herb (Wagner and Beck, 1993). It also increased nitrogen levels in older leaves by promoting the accumulation of amino acids and other nitrogenous compounds in the leaves (Jordi *et al.*, 2000). Nitrogen content of both leaves and grains were greater in the dry season than in the rainy season and it decreased with the age of the plants, It has been widely reported that primary N assimilation enzymes are down-regulated with leaf aging, while N remobilization enzymes are induced (Lerbs and Klyachko 1984; Sugiharto and Yukiko, 1992 and Masclaux *et al.*, 2000).

Carbon content in the leaves of the cowpea varieties.

Increased in carbon content was observed in all the plants given fertilizer and hormonal treatments. The highest lipid content was in the plants treated with combined 150%N+200ppm BAP followed by the single 150%N treatment. This shows that increase in the concentration of carbon in the leaves of the plants by the fertilizer might be as a result of increased nutrients due to fertilizer application, which consequently led to increased absorption of nutrients by the plants.

The properties of the soil are known to be affected by NPK fertilizer application. Yagodin (1984) reported that NPK fertilizer had predominant effect on the chemical nature and mineral composition of the soil. The hydrogen of the carboxyl groups in the soil are replaced by the various cations to yield salts known as humates which the plants absorb. The humates of univalent cations (Na⁺, K⁺, NH⁺) are water soluble compounds which are made available to the plants (Yagodin 1984; Okwu, 1999). The NPK fertilizer is converted to soluble nitrates, phosphates, sulphates or chloride which are absorbed by plants. Increased concentration of the carbon as a result of fertilizer

application was earlier on observed by many researchers, Myers (1998), Amujoyegbe *et al.*, (2001) Tolera *et al.*, (2006) and Springer *et al.*, (2005). Similar observation was also reported by Ricardo *et al.*, 1987; in their work with four selections of three amaranth species in which they were fertilized with levels of 0,30,60 and 90 kg/ha of a 12-24-12 fertilizer formulation, in which the application of fertilizer increased the carbon content of all selections. There was a general increase in the concentration of carbon in the leaves of the plants studied with increase in the level of fertilizer treatment

Table 1: Mean number of days to onset, progression and duration of senescence in the cowpea varieties, treatments and season.

Parameters	Onset of senescence	50% senescence	90% senescence	TDOP	DOS
Variety					
IT89KD288	64	81	93	102	38
Kanannado	72*	91*	102*	114*	42*
Mean	68	86	98	110	40
LSD (0.05%)	2.41	2.98	2.87	2.17	1.98
Treatments					
	Onset	50%senescence	90%senescence	TDOP	DOS
150% N	66	82	96	105	39
200ppm BAP	77*	97*	106*	120*	42
150% N+200ppm BAP	72	96	110	121	48
Control	57	69	81	92	35
Mean	68	86	98	110	41
LSD (0.05%)	5.41	7.43	4.34	3.84	2.82
Season					
	Onset	50% senescence	90% senescence	TDOP	DOS
Rainy	69*	81*	104*	117*	47*
Dry	67	91	91	102	35
Mean	68	86	98	110	41
LSD (0.05%)	1.72	2.74	4.34	1.84	1.23

TDOP- Total Death of Plant; DOS- Duration of senescence

* Statistically significant at P= 0.05

Table 2: Mean nitrogen (%) content of leaves as affected by the different varieties, treatments and seasons at different weeks.

Parameters	6 weeks	9 weeks	12 weeks	Means	LSD (0.05%)
Variety					
IT89KD-288	0.917a	0.716a	0.464b	0.699	0.0701
Kanannado	0.691b	0.619b	0.543a	0.617	0.066

Means	0.804	0.709	0.518
LSD (0.05%)	0.219	0.078	0.065

Treatment	6 weeks	9 weeks	12 weeks	Means	LSD (0.05%)
150% N	0.828b	0.693c	0.484c	0.668	0.080
200ppm BAP	0.784c	0.762b	0.602b	0.716	0.096
150% N+200ppm BAP	0.903a	0.803a	0.636a	0.781	0.095
Control	0.700d	0.575d	0.349d	0.542	0.074
Mean	0.804	0.709	0.518		
LSD (0.05%)	0.078	0.035	0.055		

Season	6 weeks	9 weeks	12 weeks	Means	LSD (0.05%)
Rainy	0.666b	0.602b	0.516a	0.595	0.0596
Dry	0.942a	0.815a	0.519a	0.759	0.0429
Means	0.804	0.709	0.518		
LSD (0.05%)	0.077	0.031	NS		

Table 3: Mean nitrogen (%) content of the grains as affected by the different varieties, treatments and seasons at harvest.

Variety	Nitrogen of Grains (%)
IT89KD – 288	3.970a
Kanannado	3.788b
Mean	3.879
LSD (0.05%)	0.134
Treatment	Nitrogen of Grains (%)
150% N	4.033b
200ppm BAP	3.724c
150%N+200ppmBAP	4.355a
Control	3.405d
Mean	3.879
LSD (0.05%)	0.133

Season	Nitrogen of Grains (%)
Rainy	3.788b
Dry	3.970a
Mean	3.879
LSD (0.05%)	0.089

Table 4: Mean carbon (mg/100g) content of the two cowpea varieties.

Parameters	6 weeks	9 weeks	12 weeks	Mean	LSD(0.05%)
Variety					
IT89KD-288	41.44a	40.87a	38.07b	40.12	1.480
Kanannado	41.19a	40.54a	39.01a	40.58	1.501
Mean	41.81	40.70	38.79		
LSD (0.05%)	NS	NS	0.810		
Treatments					
150% N	42.98b	40.23c	38.51c	40.57	1.998
200ppm BAP	42.39c	41.66b	40.36b	41.46	NS
150%N+200ppmBAP	43.31a	42.87a	41.85a	42.67	NS
Control	39.31d	37.33d	34.44d	37.03	1.826
Mean	41.81	40.70	38.79		
LSD (0.05%)	1.513	1.311	1.762		
Season					
Rainy	40.65a	40.31a	39.65a	40.20	NS
Dry	43.31a	40.75a	37.93b	40.66	0.920
Mean	41.81	40.70	38.79		
LSD (0.05%)	NS	1.261	0.903		

CONCLUSION

In conclusion the combined liquid nitrogen fertilizer and BAP regulated the onset of senescence had effect on Nitrogen mobilization and increased yield in the two cowpea varieties.

From the findings of the study the following could be recommended:

1. The use of liquid Nitrogen fertilizer could be recommended to farmers for improving growth and yield in photosensitive cowpeas.
2. The use of BAP could be recommended for use as spray to delay senescence and nutrient remobilization and increase the chlorophyll content of the cowpea.

3. Nitrogen fertilizer in combination with BAP could be used to extend the reproductive period and the photosynthetic efficiency of the plant which lead to increase in yield as well as delay senescence in the photosensitive cowpea studied.

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