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Full Length Research Paper

Effect of benzyl amino purine (BAP), coconut milk (CM) and manure applications on leaf senescence and yield in photoperiod sensitive cowpea variety (Kanannado)

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The relationship between leaf senescence in cowpea [*Vigna unguiculata* (L) Walp.] and yield was studied in pot grown population of a local variety, kanannado. Treatments comprising of farm yard manure (single and double application), 200 ppm benzyl amino purine (BAP), 15% coconut milk (CM) and a combination of both manure and the hormones were also applied to determine their effects on senescence and yield. The experiment was carried out at the International Institute of Tropical Agriculture (I.I.T.A), Kano station, Nigeria. Chlorophyll level was increased by all the treatments with greater increase in the 200ppm BAP and 15% CM treated plants. The level of chlorophyll also decreased with increase in age of the cowpea plants. Senescence commenced earlier and progressed faster in the manure treated plants while it was significantly delayed by 200ppm BAP and 15% CM. Fodder and grain yields were greater in all the treated cowpea plants compared with the control. Interactive effects were observed in the combined treatments (manure +15% coconut milk and manure +200 ppm BAP) as chlorophyll increased above that of manure and control plants. Also senescence process was delayed and yield was increased. The study suggests that leaf senescence in the photoperiod sensitive cowpea could be delayed by application of 200 ppm BAP, 15% CM and a combination of manure +15% CM and manure +200 ppm BAP and the delayed senescence resulted in increase in yield.

Key words: Farmyard manure, benzyl amino purine, coconut milk, leaf senescence, yield.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L) Walp], a protein rich (23 - 25%) leguminous crop is mainly cultivated for its dry seeds, fodder and to a lesser extent for ground cover or green manure (Singh et al., 1997).

Cowpeas show a great variation in the start and end of the reproductive period. Some cultivars flower within 30 days from sowing and are ready for dry harvest 25 days later. Others take more than 100 days to flower and between 210 and 240 days to mature. Generally, genotypes that flower early have shorter blooming periods, that is the number of days for which new flowers continue to open is shorter than those genotypes that flower later (about 18 and >30 days respectively) (Ishyaku and Singh, 2001). Cowpea responds to photoperiod in a manner typical of quantitative short day plants (Patel and Hall, 1990). The local varieties, because of their short day requirement flower early when grown with irrigation in the dry season which is characterized by short day length (Mukhtar and Singh, 2005). The early flowering advances senescence and early death of the crop. This reduces both fodder and grain yields (Green and Jones, 1982).

Senescence is a highly regulated complex process that occurs as part of plant development which ultimately leads to death of plant (Taiz and Zeiger, 2002). In annuals like cowpea, the whole plant dies after a limited period of growth and completion of reproduction (Green and Jones, 1982). Photoperiod and temperature are two environmental factors that govern senescence and death in plants. Short day length accelerates flower initiation in photoperiod sensitive cowpeas and death (Ismail and Hall, 1998; Mukhtar and Singh, 2005) . Senescence causes substantial loss in total grain yield because most

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Table 1. Effect of manure, Benzyl amino purine, (BAP) and coconut milk treatments on chlorophyll content of pho-operiod sensitive cowpea variety (kanannado) planted in the dry season.

Treatment	Changes in chlorophyll (mg chlorophyll/g tissue) content with time (weeks) kanannado			
	3	6	9	12
Manure x1	1.50	1.44	0.98	0.37
Manure x2	1.47	1.24	0.95	0.46
200ppm BAP	1.27	1.21	0.86	0.55
15% coconut milk	1.36	1.35	1.33	0.78
Manure +200ppm BAP	1.42	1.27	1.01	0.52
Manure +15% Coconut milk	1.44	1.37	1.09	0.60
Control	1.10	1.01	0.66	0.31
Mean	0.12	0.18	0.09	0.02
LSD $_5$%	0.12	0.18	0.09	0.02

cowpea plants die after producing the first flush of pods (Ismail and Hall, 1998). Nutrient mobilization by the seeds from the leaves is one of the causes of sene-scence in plants as it results in starvation or some other nutrients deficiency that causes death (Molisch, 1982). In this study manure was applied to enrich the soil in order to determine the effect of increased soil nutrition in the regulation of the onset, progression and duration of sene-scence in cowpea.

The hormone, cytokinin is associated with senescence as it is shown to delay leaf senescence when applied by attracting nutrients from other parts of the leaf to the treated area (Pruitt, 1983). Consequently the effect of benzyl amino purine (BAP) and coconut milk (crude source of cytokinin) in the regulation of the onset and duration of senescence was also investigated in this study. The effect of interaction of manure and cytokinin was also studied. The aim was to investigate whether application of cytokinin and manure to cowpea would delay leaf senescence and total death of the plants thereby increasing the fodder and grain yields of the crop during the dry season. This is because flowering and senescence are early in the dry season and yield is lower when compared with rainy season planting (Mukhtar and Singh, 2005, Mukhtar, 2007). Also senescence studies carried out have not attempted to relate it to yield.

MATERIALS AND METHOD

The experiment was carried out at the International Institute for Tropical Agriculture (IITA), Kano station, Nigeria. Kano is situated at latitude 12°03'N, Longitude 8°34 E and altitude 486.5 m (1595 ft). Kano is characterized by two seasons, the rainy (May to September) and the dry season (October to May) average minimum and maximum temperature during the dry season, 2007 was 19 and 37°C. Relative humidity during the dry season was 81% maximum and 49% minimum. Photoperiod ranged from 12.5 h to 13.4 hd^{-1} in the dry season. The cowpea variety selected for the study was Kanannado which is a local variety and it was collected from I.I.T.A, Kano station. Plastic pots of 250 mm diameter were used in the study. They were filled with top loamy soil and well watered for two days before planting. The seeds were directly sown in the prepared pots at the rate of five seeds per pot (after surface treatment with fungicide, Apron plus). After germination, they were thinned to two plants per pot. The pots were properly labeled according to the type of treatment using white and green plastic tags. Planting was done in the dry season on 24th October, 2007.

There were seven treatments including the control. These were; farm yard manure collected from IITA and used at two levels, single application at planting and double application, at planting and at flower bud initiation stages. The other treatments were, foliar application of 200 ppm benzyl amino purine (BAP) which is a cytokinin and 15% coconut milk (cm) which is also a crude source of cytokinin in three doses; 3 weeks, bud initiation and flowering stages. Coconut milk was used because it is available locally and affordable. There were also combined treatments to check for possible interaction between manure and the hormone treatments. Consequently manure was applied at planting stage and was followed by the foliar spray with 200 ppm benzyl amino purine or coconut milk at three doses as described earlier. There were ten replications for each treatment. Watering and weeding were carried out regularly. The pots were arranged in completely randomized design.

Data were collected on chlorophyll content at three weeks interval. Also number of days taken to 50% senescence, 90% senescence and complete death of plant was recorded. From the data obtained, the duration of senescence in the different treated and control plants were estimated. Analysis of variance was carried out to determine significant differences between the treatments and least significant difference (LSD) test at P 0.05 was used to separate the means.

RESULTS AND DISCUSSION

Table 1 shows the effect of the different treatments on changes in chlorophyll content of the photoperiod sensitive cowpea. Chlorophyll content was greater at 3 weeks after planting in all the treated and control plants. The chlorophyll content decreased with increase in number of weeks after sowing and it was least at 12 weeks (Table 1). Watson et al. (1988) reported that senescence in green leaf is brought about by loss of chlorophyll. Degree of chlorophyll loss gives an idea of the actual cell death. Chen et al. (1991) in their studies on senescence in rice leaves observed that the level of chlorophyll decreased with increasing age and that chlorophyll loss is commonly used as a prime indicator of leaf senescence. In the present experiment, all the treatments significantly (P 0.05) increased the chlorophyll content of the leaves when compared with the control. Comparison of the treatments revealed that at 3 weeks after planting, manure treated plants (manure x1 and manure x2) had greater chlorophyll contents (1.50 and 1.47 mg chlorophyll/g tissue) followed by the combined treatments of manure + 15% coconut milk (1.44 mg chlorophyll/g tissue) and manure + 200 ppm BAP (1.42 mg chlorophyll/g tissue). However, by 12 weeks after sowing, cowpea plants treated with 15% coconut milk had the highest chlorophyll content

Table 2. Effect of manure, benzyl amino purine (BAP) and coconut milk treatments on the onset and progression of senescence and yield in photoperiod cowpea variety kanannado.

Treatments	Progression of senescence (days)				
	Onset	50%	90%	Total death	Duration of senescence (days)
Manure x1	64.8	71.8	80.9	86.4	21.6
Manure x2	62.8	71.5	81.4	87.4	24.6
200ppm BAP	69.6	79.0	88.0	96.0	26.4
15% coconut Milk	71.6	82.3	91.8	98.6	27.0
Manure +200ppm BAP	66.2	75.8	85.8	91.6	25.4
Manure +15% Coconut milk	65.5	76.0	85.8	91.9	25.4
Control	65.5	75.4	84.8	90.0	24.5
Mean	66.6	76.0	85.5	91.8	25.0
LSD $_5$%	3.287	2.378	2.515	2.356	1.830

(0.78 mg chlorophyll/g tissue). This was followed by the combined treatments of manure +15% coconut milk (0.60 mg chlorophyll/g tissue) and 200 ppm BAP (0.55 mg chlorophyll /g tissue) while the manure treated plants had the lowest amount (0.37 and 046 gm chlorophyll /g tissue) (Table 1). Organic manure was observed to significantly increase the chlorophyll content of leaves of spring barley *Hordeum vulgare* (Ofosu and Leitch, 2009).

Atanasiu et al. (1983) reported similar increase in chlorophyll content of *chlorella vulgaris* 157 upon treatment with kinetin. Cytokinin inhibited chlorophyll loss during aging of wheat chloroplasts (Misra and Biswal, 1980). The results of the effect of the treatments on the onset, progression and duration of senescence are presented in Table 2. All the treatments delayed the onset of senescence in the cowpea plants with the exception of the manure treatments (manure x1 and manure x2) which hastened it when compared with the control. The difference was however significant only in the hormone treatments (15% coconut milk and 200 ppm BAP). Similar observations were made with respect to progression of senescence (days to 50%, 90% senescence and total death f the plants), that is, manure treatments accelerated the process while 15% coconut milk and 200 ppm BAP significantly ($P \leq 0.05$) delayed the process (Table 2). The results indicated that of all the treatments 15% coconut milk and 200PPm BAP had greater effect in delaying onset and progression of senescence, followed by the combined treatments (manure +15% coconut milk and manure +200ppm BAP), thereby increasing the vegetative life span of the treated plants. Similarly, the duration of senescence, from onset to total death was significantly shorter (21.6 days) in the single manure application when compared with the control and all the other treatments. The other treatments however increased the duration of senescence compared to control (Table 2). These treatments therefore increased the reproductive period of the treated plants.

The observation that the manure treatments resulted in earlier senescence and death of plants is supported by the explanation of chowdhury and Johry (2003) that plants that bolted early also senesced early, accummulated less chlorophyll but produced more fruits. The degree of chlorophyll loss indicates the extent of cell death as reported by Watson Spare (1988).

Manure is not known to delay senescence (Whalen et al., 2000; Maerere et al., 2001; Vanek et al. (2003) but on the other hand, Mazid (1993) and Seobi et al. (2005) reported hat application of cow dung to tumeric plant caused the plants to remain green for a longer period. The observation that the onset of senescence was delayed by 15% coconut milk and 200 ppm BAP treatments (Table 2) suggests that, the duration of vegetative growth was increased. Also that number of days to total death of the plants was increased by these two treatments implies that the reproductive period was longer (Table 2).

Benzyl amino purine (BAP) is known to have a retarding effect on the process of senescence. Treated leaves remained green many days longer than those untreated. This effect has been suggested to be as a result of BAP activity to induce increased flow of material through the vascular tissues to its own location and to prevent the transport of substances away from it. In fact, physiological studies suggest that the internal BAP level drops with progression of leaf senescence (Smith and Watson, 1988). Smith and Watson (1988) suggested that spraying of BAP delay the appearance of reproductive structures in plants and this serves as a means of minimizing early senescence. The combination of manure +15% coconut milk and manure +200ppm BAP also delayed the onset of senescence as well as slowed down the progression of senescence. This suggests that there was interaction between manure and the hormones. Consequently, 15% coconut milk and BAP applied singly or in combination

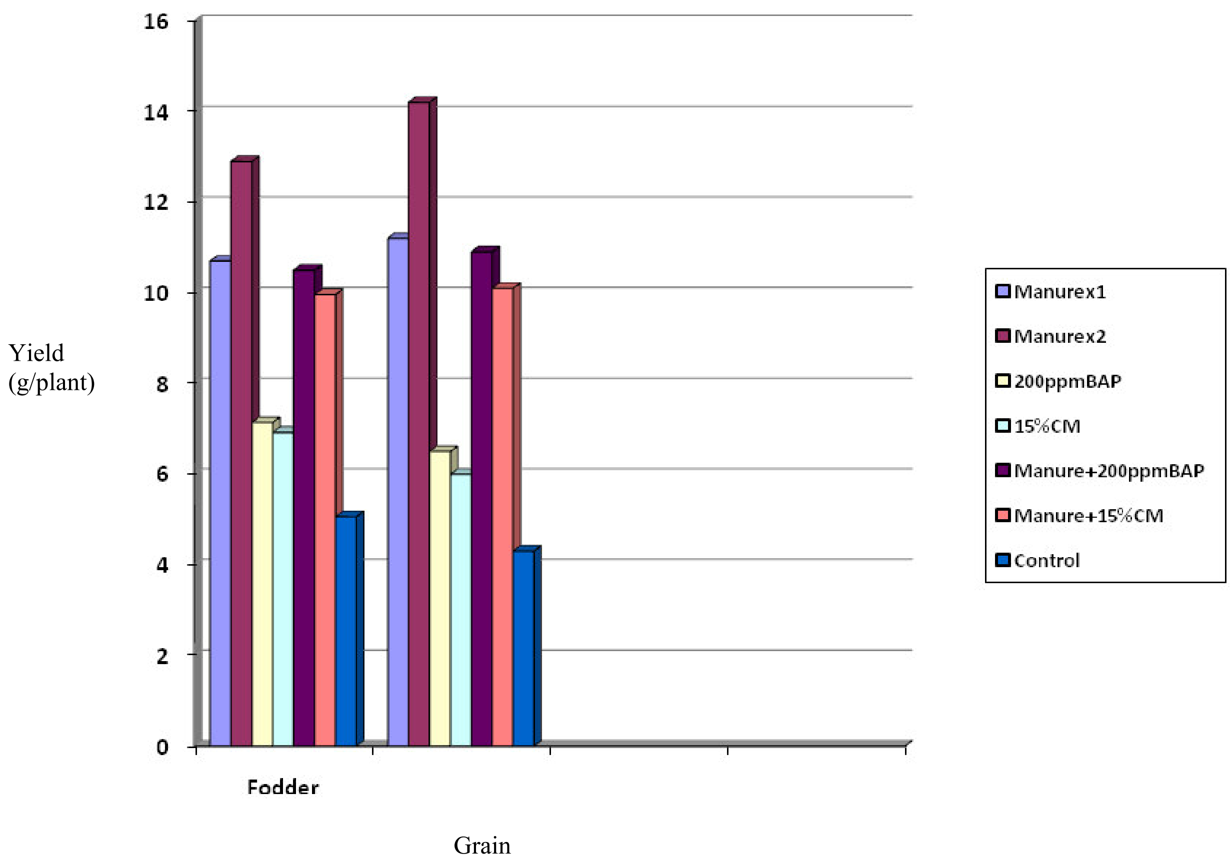

Figure 1. Effect of manure, benzyl- amino purine and coconut milk treatments on fodder and grain yields (g) per plant.

with manure significantly delayed cell death when compared with the control and the manure treatments. This resulted in a delay in the initiation and progression of senescence.

Both fodder and grain yield were significantly (P 0.05) increased by all the treatments when compared with the control (Figure 1). The manure treatments (double and single applications) resulted in greater fodder (12.9 and 10.7 g respectively) and grain (14.2 and 11.2 g respec-tively) yields followed by the combined treatments of manure + BAP (10.5 and 10.9 g) and manure +15% coconut milk (9.96 and 10.1 g) for both fodder and grain yields respectively (Table 3) . Manure is known to improve soil productivity and fertility and this improves yield and quality of crops (Whalen et al., 2000; Maerere et al., 2001; Vanek et al., 2003). Nour (1998) and El-Kholy et al. (1999) found that, application of farm yard manure sig-nificantly increased grain yield in rice compared with the control. Vanek et al. (2003) reported that regular appli-cation of manure to root crops leads to higher yield. Even though 200 ppm BAP and 15% coconut milk delayed initiation and progression of senescence thereby pro-longing both vegetative and reproductive life span of the treated plants, fodder and grain yields were however lower in comparison with the other treatments except the control. This could be because cytokinins are known to stimulate cell division but not growth (Taiz and Zeiger, 2002). Yield in cowpea on the other hand is related to growth that is the number of internodes at which pods could be set. Therefore plant size and consequently number of internodes at flowering influence yield in cow-pea (Mukhtar, 2007). Delayed leaf senescence enhances the extent of plant survival but may reduce first flush grain yield (Ehler et al., 2000). In the present experiment in cowpea many plants died after producing the first flush of pods. This may have caused the reduction in the yield since the second flush yield will be proportional to the number of plants surviving to produce the second flush (Ismail and Hall, 1998). Fodder and grain yields were however greater when 200ppm BAP and 15% coconut milk were applied to manure treated plants which may be due to the interaction effect.

Conclusion

The study showed that the manure treatments especially when applied twice had lower chlorophyll contents, en-hanced senescence and early death of the cowpea plants. However, both fodder and grain yields were hig-her in these treatments. Hormone treated plants had the highest chlorophyll. Leaf senescence was retarded by the application of the hormone, 200 ppm benzyl amino purine

(BAP) and 15% coconut milk. The extent of delay was more when the plants were treated with 15% coconut milk. Fodder and grain yields were also increased when compared with the control. The combined treatments (manure + BAP and manure +15% coconut milk) also increased chlorophyll content, and increased fodder and grain yields above the control and single hormone treatments. The findings of this study, therefore suggest that delayed leaf senescence could increase fodder and grain yields of the photosensitive cowpea variety when grown in the dry season (with irrigation) which is characterized by short day length.

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