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Agronomy

Forage Cowpea (*Vigna unguiculata*) Seed Yield and Seed Quality Response to Foliar Application of Bio-regulators

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Abstract

Heat stress could be one of the major constraints for limiting yield and quality of legumes in many parts of the world. Foliage applied bio-regulators induce thermo-tolerance and flowering in plants under temperature stress conditions. So, we assessed the effectiveness of four foliage applied bio-regulators starting from flower initiation on growth, seed yield and germination parameters on two cultivars of forage cowpea (*cv.* CL 367 and Cowpea 88) with different doses *viz*. sodium benzoate (100 and 150 µg mL^{-1}), salicylic acid (50 and 100 µg mL^{-1}), $CaCl_2$ (0.5 and1.0%) and KNO_3 (1.0 and 2.0%). Cultivar CL 367 recorded significantly higher SPAD value, pods plant^{-1}, seed yield, harvest index, and seed germination parameters than Cowpea 88. Bio-regulators sprays on crop improved the seed yield by 12.9-32.7%. Salicylic acid (50 µg mL^{-1}), KNO_3 (2.0%) and $CaCl_2$ (1.0%) foliar sprays increased seed yield by 32.7%, 27.5% and 26.7%, respectively, over control. Seeds produced from 50 µg mL^{-1} salicylic acid treatment were of better quality with improved germination, shoot length, root length and seedling vigor index. Our results suggest that three foliar sprays of 50 µg mL^{-1} of salicylic acid starting from flowering at weekly interval can increase the seed yield and quality of cowpea. Improvement in seed yield and its quality with foliar bio-regulators will certainly increase the area under this fodder crop and will be helpful in sustaining livestock production in developing countries.

Highlights

- Cultivar CL 367 recorded better growth, seed yield and seed quality than C 88.
- Three foliar applications of 50 µg ml^{-1} salicylic acid starting from flower initiation at weekly interval recorded 32.7% higher seed yield than control.
- Foliar sprays of bio-regulators improved germination, shoot length, root length and seedling vigor index of produced seed.

Keywords: Bio-regulators, cowpea, potassium nitrate, salicylic acid, seed yield, seed quality

Cowpea *(Vigna unguiculata* L.Walp.) is a quick growing and high yielding crop fed to livestock and also makes a valuable contribution towards human food in tropical and subtropical parts of the world (Kumar *et al.* 2014; Sarma *et al.* 2014). However, non-availability of good quality cowpea seed is a serious problem in developing countries throughout the world (Biemond *et al.* 2012) leading to little availability of good quality fodder to animals which is one of the major reasons for low milk productivity in South Asian countries (Suresh *et al.* 2012). The scarcity of good quality green legume fodder is more serious during the summer months when dairy animals require the most for their growth and development compared with during the winter season.

Although good agronomic practices like optimum time of sowing, seed rate, fertilization, weed control and harvesting have significant positive influence on seed yield, however, environmental conditions like temperature, soil moisture and relative humidity during the reproductive phase play an important role for seed formation and development in cowpea (Kiari *et al.* 2011). High temperatures and prolonged dry spells during reproductive phase can cause male sterility, anther dehiscence and excessive floral abscission leading to poor pod setting as well as seed yield of cowpea (Ahmed *et al.* 1992; Mutters and Hall, 1992; Thuzar *et al.* 2010). High night temperatures at flowering stimulate respiration, damage the cell membranes and reduce net photosynthesis and the transfer of photo assimilates into the seed (Wahid *et al.* 2007; Hayat *et al.* 2009; Kumar *et al.* 2013). One degree increase in average daily minimum night temperature above 16.5°C one week prior to the first appearance of flower in cowpea reduced the number of pods by 11.5% and grain yield by 13.5% in California (Ismail *et al.* 2000). Other studies demonstrated that night temperature above 17°C particularly during flowering adversely affects the pod setting and seed yield in cowpea (Ajetomobi and Abiodun, 2010). Night temperature during summer season as well as at time of reproductive phase (August-September) of cowpea in North-West India remains > 20°C (around 24°C) (Prabhjyot-Kaur *et al.* 2006) which may adversely affect the flowering and seed setting in cowpea.

Reduction in the yield of field crops can be managed by use of foliar bio-regulators as these can modify plant physiological/biochemical processes during biotic and abiotic stresses (Kumar *et al.* 2013). Bio-regulators application on crop enhances the number of flowers, reduces pod drop, improve endogenous content of mineral ions and provide tolerance during heat stress conditions (Wahid *et al.* 2007; Kumar *et al.* 2013; Bons *et al.* 2015). Bio-regulators such as sodium benzoate and salicylic acid are known to stimulate photosynthetic machinery, improves chlorophyll and protein synthesis, inhibit ethylene production, delay senescence and may induce tolerance to plants to heat stress which helps to increase seed yield in number of crops mainly due their action in multi facets way (Kumar *et al.* 2013; Bons *et al.* 2015). $CaCl_2$ and KNO_3 are osmo-protectants which improve photosynthesis, cell division, cell elongation and influence the crop growth through its effect on water uptake, root growth, maintenance of turgor and transpiration in cells, and activate many enzymes under environmental stress conditions (Wahid *et al.* 2007).

Foliar application of salicylic acid also helps in uptake Ca^{2+} from soil which has important role in heat tolerance (Waraich *et al.* 2012) and thus improve seed yield in number of crops (Hayat *et al.* 2009; Hayat *et al.* 2010; Kumar *et al.* 2013; Bons *et al.* 2015). $CaCl_2$ and KNO_3 when applied on the foliage of crops raise osmotic pressure in the cytoplasm, stabilize proteins and membranes when salt level or temperature is unfavorable and thus play important roles in the adaptation of cells to various adverse environmental conditions (Wahid *et al.* 2007; Kumar *et al.* 2013).

With the passage of time, climate change is likely to accentuate the frequency and intensity of heat stress and drought which definitely will affect the seed yield and quality of crops particularly in tropical and subtropical areas of the world. We therefore hypothesized that under such conditions, foliar sprays of bio-regulators will be equally effective, easy and economical approach for increasing the seed yield of crops. Spraying cowpea plants with an appropriate bio-regulator at low doses may overcome these adverse effects. Improving the seed productivity of cowpea under moderately high temperature has practical relevance. However, there is lack of comprehensive information available in the literature on the effect of different bio-regulators and their required concentration on seed yield and quality of forage cowpea vulnerable to heat stress, particularly during reproductive phase. Furthermore, legume species and their cultivars may differ in their response to the application of bio-regulators (Kumar *et al.* 2013). The present study was therefore, carried out to investigate the effect of different bio-regulators (e.g. salicylic acid, sodium benzoate, KNO_3 and $CaCl_2$) on growth, yield attributes, seed yield and seed quality (germination parameters) of two cultivars of fodder cowpea.

Materials and Methods

Field experiments were conducted during summer seasons of 2008 and 2009 in Ludhiana, Punjab, India at the Fodder Farm of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (30°56′ N, 75°52′ E, 247 m above sea level) from August to November. The surface (0-15 cm) soil of the experimental field was sandy loam in texture (*Typic Ustochrept*) having pH 7.2, 0.25% Walkley and Black carbon, 11.3 kg $NaHCO_3$-extratable Olsen P ha^{-1} and 289 kg K ha^{-1} NH_4OAc-extractable K. Total amount of rainfall received during the crop seasons (second week of August to mid of November) was 424.1 mm and 213.4 mm during the year 2008 and 2009, respectively. Mean maximum and minimum monthly air temperatures recorded were 34.3°C and 12.6°C during the month of August and November and 34.7°C and 11.3°C in September and October months during the year 2008 and 2009, respectively. The minimum temperature during flowering and reproductive phase (38th and 39th standard week) of crop ranged from 20.9°C to 21.9°C and 24.3°C to 24.7°C during the second week of September to end of September in the year 2008 and 2009, respectively. The treatments included combinations of two recommended cultivars (CL 367 and Cowpea 88) of fodder cowpea and four combinations of bio-regulators and their concentrations namely, sodium benzoate (100 and 150 µg mL^{-1}), salicylic acid (50 and 100 µg mL^{-1}), $CaCl_2$ (0.5 and 1.0%) and KNO_3 (1.0 and 2.0%).

An additional untreated control was also included in the study. The experiment was laid out in a factorial randomized block design with three replications. The experimental crop was sown in lines on August 7 and August 8 in the year 2008 and 2009 respectively keeping the row to row distance 30 cm in well prepared seed bed using 20 kg seed ha^{-1} for genotype CL 367 and 40 kg seed ha^{-1} for genotype Cowpea 88. Before sowing, seeds of both the cultivars were treated with bavistin (Carbendazim 50% WP) at 2 g kg^{-1} of seed for protecting the crop from fungal diseases. A basal dose of 20 kg N ha^{-1} (as urea) and 24 kg P ha^{-1} (as single super phosphate) was applied at sowing. Stomp (Pendimethalin) 30 EC at 2 L ha^{-1} was applied immediately after sowing of the crop during both the year to control the weeds. A total of 4 irrigations in 2008 and 5 irrigations in the year 2009 were given to the crop during the growing seasons. To control crop damage due to aphid and jassid attack, 500 mL ha^{-1} Malathion (Organophosphate insecticide) was sprayed on September 14 during 2008 and September 19 during 2009.

Three foliar sprays of the bio-regulators were applied at weekly interval starting from flower initiation. Cultivar Cowpea 88 exhibited flower initiation on September 18, whereas in cultivar CL 367 flowering started from September 24 during the year 2008. The corresponding dates for flower initiation in cultivar Cowpea 88 and CL 367 during the year 2009 were September 21 and September 26, respectively. Thereafter, seed crop was harvested from 15 m^2 area, on November 11, 2008 and November 13, 2009 when the pods turned yellow and was allowed to dry in the field for one week for easy threshing and storage. The growth parameters such as SPAD leaf chlorophyll index (from 65 days old crop), plant height from base to top of twine and total fresh weight of five randomly selected plants from each experimental plot was measured.

Similarly seed yield and yield attributes *viz.* number of pods plant^{-1}, pod length, seeds pod^{-1}, seed yield plant^{-1}, 100-seed weight were also recorded. Seed and stover yields were computed on oven-dry weight basis and converted to tonne ha^{-1}. The harvest index was computed by dividing the seed yield by biological yield (grain + stover yield). For assessing seed quality (germination parameters), a sub-sample of 20 seed was collected from the bulk seed within each plot. The collected seeds were surface sterilized with 0.1% mercuric chloride for two minutes, washed thoroughly and then kept in petri dish at an equal distance in BOD incubator at 28°C for 10 days. Various germination parameters such as % germination, shoot length, root length and seedling vigor index were measured. The data analyzed using analysis of variance (ANOVA) by using IRRISTAT version 92 (IRRI, 1992). The crop data were analyzed keeping varieties as factor A and foliar treatments as factor B in factorial randomized block design. The comparison of

Table 1. Effect of different bio-regulators on growth and yield attributes of two cowpea cultivars (data averaged over two years)

Treatments	SPAD Chlorophyll Value	Plant height (cm)	Fresh weight plant^{-1} at harvest (g)	Pods plant^{-1}	Pod length (cm)	Seeds pod^{-1}	Seed yield plant^{-1} (g)	100-seed weight (g)
Cultivars								
CL 367	52.5a	184.5b	178.6b	26.8a	14.4a	13.6b	33.6a	10.4b
Cowpea 88	44.8b	198.6a	207.3a	20.4b	14.8a	14.4a	30.8b	13.9a
Foliar bio-regulators								
Control	40.7c	165.8d	164.5d	18.5d	12.8c	13.1b	27.3c	11.2b
Sodium benzoate (100 µg mL^{-1})	42.5c	177.8c	175.2cd	20.1cd	14.3b	13.6b	30.0bc	11.6b
Sodium benzoate (150 µg mL^{-1})	46.5bc	185.7c	183.6c	22.0c	14.6b	14.0b	30.7b	11.9b
Salicylic acid (50 µg mL^{-1})	55.5a	209.4a	212.6a	28.1a	15.7a	15.0a	36.1a	13.3a
Salicylic acid (100 µg mL^{-1})	50.1b	195.4bc	200.0b	25.1b	14.6b	14.0b	31.5b	12.2b
$CaCl_2$ (0.5%)	50.4b	192.6bc	193.1bc	24.5bc	14.4b	13.8b	32.3b	12.1b
$CaCl_2$ (1.0%)	51.8ab	198.9ab	202.9ab	25.6ab	14.8ab	14.1ab	34.2ab	12.6ab
KNO_3 (1.0%)	48.1b	197.4b	197.6b	22.8bc	14.5b	13.9b	31.3b	12.0b
KNO_3 (2.0%)	52.5ab	203.0ab	206.8ab	26.3ab	15.3ab	14.6ab	34.7ab	12.7ab

Values followed by same lower-case letters within a column are not significantly different at p = 0.05.

treatment means was made by least significant difference (LSD) at $p = 0.05$.

Results and Discussion

No significant cultivar × bio-regulator treatment interaction effects were observed in different yield parameters, seed yield and seed quality of cowpea in both years of the study. Therefore, main effects of cultivar and foliar treatments are presented and discussed. Similarly, there were no significant year × treatment and year cultivar interaction on different crop parameters.

Effect of Cultivars

Cultivar CL 367 had significantly higher (17.2%) SPAD value and recorded significantly more (31.4%) number of pods plant^{-1} than in Cowpea 88 (Table 1). However, cultivar Cowpea 88 had recorded significantly taller plants (198.6 cm *vs* 184.5 cm) having more fresh weight plant^{-1} (207.3 g *vs* 178.6 g) at harvest and significantly more seeds pod^{-1} than the CL 367 cultivar. Differences in plant parameters (like SPAD value, plant height and fresh weight plant^{-1}) observed in the two cultivars of cowpea may be related to their genetic structure as reported by El-Naim and Jabereldar (2010). Seed yield plant^{-1} was 9.0% higher in CL 367 than Cowpea 88 cultivar due to more numbers of pods plant^{-1}. On the other hand, 100-seed weight was significantly higher in Cowpea 88 than in CL 367 cultivar. Cultivar CL 367 recorded significantly higher total seed yield (37.6%) and harvest index (32.2%) compared to Cowpea 88 (Table 2).

Similarly, differences among genotypes for various yield parameters such as pods plant^{-1}, seeds pods^{-1}, seed yield plant^{-1} and 100-seed weight could be attributed to the intrinsic ability of different cultivars to access growth resources and their expression in terms of yield attributes and seed yield (Antwi *et al.* 2012). The better performance of CL 367 cultivar in terms of seed yield, stover yield and harvest index was due to higher SPAD value (Table 1) indicating higher chlorophyll content and photosynthesis, more generation of photo assimilates which in turn led to formation of more pods and more seed yield plant^{-1}. Also late flowering by 5-7 days in cultivar CL 367 might had provided more time for manufacturing of photo-assimilates which increased seed yield compared to Cowpea 88. Ngodi and Dauda (2010) also recorded higher seed yield plant^{-1} in cultivar Karagoz 86 due to higher number of pods plant^{-1}. Differences in genetic characters of cultivars and their expression might be the reasons for better seed quality parameters such as germination percentage, shoot and

Table 2. Effect of different bio-regulators on seed yield, stover yield, harvest index and seed quality parameters of two cowpea cultivars (data averaged over two years)

Treatments	Seed yield (t ha^{-1})	Stover yield (t ha^{-1})	Harvest index	Seed quality parameters			
				Germination percentage	Shoot length (cm)	Root length (cm)	Seedling vigor index
Cultivars							
CL 367	1.61a	8.84a	15.6a	88.0a	14.8a	12.4a	2404.4a
Cowpea 88	1.17b	8.76a	11.8b	86.1a	13.1b	11.8b	2292.0b
Foliar bio-regulators							
Control	1.16d	7.15c	13.9a	82.1b	12.5b	10.8c	1917.8c
Sodium benzoate (100 µg mL^{-1})	1.31c	8.34b	13.6a	84.1b	13.9a	11.5bc	2155.6b
Sodium benzoate (150 µg mL^{-1})	1.38bc	8.50b	13.8a	86.7b	14.2a	11.8b	2261.1b
Salicylic acid (50 µg mL^{-1})	1.55a	9.52a	13.9a	93.7a	14.7a	13.3a	2618.4a
Salicylic acid (100 µg mL^{-1})	1.42b	9.16ab	13.5a	86.7b	13.9a	12.3b	2329.7b
$CaCl_2$ (0.5%)	1.39bc	9.02ab	13.5a	87.1b	13.8a	11.7b	2258.1b
$CaCl_2$ (1.0%)	1.47ab	9.14ab	13.9a	89.2ab	14.0a	12.4ab	2374.1b
KNO_3 (1.0%)	1.39bc	8.97ab	13.3a	84.6b	14.2a	12.0b	2210.8b
KNO_3 (2.0%)	1.48ab	9.25a	13.8a	89.1ab	14.6a	12.8ab	2442.5ab

Values followed by same lower-case letters within a column are not significantly different at $p = 0.05$.

root length and seedling vigor index (SVI) in CL 367 compared with Cowpea 88 (El Naim and Jabereldar 2010). Both the cultivars did not differ significantly with respect to their mean pod length and stover yield (Table 1 and 2).

Effect of Bio-regulators

All bio-regulators recorded significantly higher SPAD values, plant height, fresh weight plant^{-1}, pods plant^{-1}, seeds pod^{-1}, pod length, seed yield plant^{-1}, 100-seed weight, total seed yield and stover yield of cowpea (Table 1 and 2) as compared to control, while maximum values were noted in 50 µg mL^{-1} salicylic acid. Salicylic acid at 50 µg mL^{-1} increased mean number of pods plant^{-1} and pod length over control and was at par with foliar application of 2.0% KNO_3 and 1.0% $CaCl_2$. Foliar sprays of different bio-regulators on cowpea recorded 11.7 - 22.7% increase in the pod length than control treatment. Application of salicylic acid (50 µg mL^{-1}), KNO_3 (2.0%) and $CaCl_2$ (1.0%) recorded 14.5%, 11.5% and 7.6% increase in number of seeds pod^{-1} over control, respectively. Foliar application of 50 µg mL^{-1} salicylic acid showed maximum increase in seed yield plant^{-1} and was closely followed by 2.0% KNO_3 and 1.0% $CaCl_2$ among all bio-regulators (Table 1). The mean increase in seed yield plant^{-1} over control with salicylic acid (50 µg mL^{-1}), 2.0% KNO_3 and 1.0% $CaCl_2$ was 32.2%, 27.1% and 25.2%, respectively. Foliar sprays of sodium benzoate at 100 and 150 µg mL^{-1} showed no significant increase in mean seed yield plant^{-1} over control. Mean (averaged over two years) 100-seed weight was however, significantly higher in case of salicylic acid (50 µg mL^{-1}), KNO_3 (2.0%) and $CaCl_2$ (1.0%) compared to control.

Salicylic acid increases chlorophyll *a* and *b,* carotenoid content and Rubisco activity thereby increases the rate of CO_2 assimilation in plants leading to improved growth traits (Hayat *et al.* 2009), photosynthetic activity (Kumar *et al.* 2013; Bons *et al.* 2015), cell elongation and mitosis (Wahid *et al.* 2007) which possibly caused increase in plant height and fresh weight plant^{-1} of cowpea in our study.

The increased availability of Ca^{2+} from $CaCl_2$ increase chlorophyll content and photosynthesis leading to taller plants than control (Wahid *et al.* 2007; Rab and Haq, 2012). Similarly, foliar application of KNO_3 increase synthesis and translocation of photo-assimilates (Kumar *et al.* 2013), which might be responsible for increase in SPAD values, plant height and fresh weight plant^{-1} of cowpea. Similar trend was also recorded with pods plant^{-1}. Consistent with our study, Sharma and Kaur (2003) also reported similar increase in yield contributing characters with foliar sprays of 50 µg mL^{-1} of salicylic acid in soybean (*Glycine max*). Rab and Haq (2012) reported that spraying strawberry plants with 0.5% $CaCl_2$ solution significantly increased yield components and fruit yield over control. However, no comprehensive information is available in the literature on the role of foliar bio-regulators on growth and seed yield of fodder cowpea.

All bio-regulators showed significant increase in seed and stover yield of cowpea over control (Table 2). Maximum total seed yield was recorded in foliar application of salicylic acid at 50 µg mL^{-1}, which was statistically at par with 1.0% $CaCl_2$ and 2.0% KNO_3, but was significantly higher than the other foliar bio-regulators. On two-year average, salicylic acid (50 µg mL^{-1}), $CaCl_2$ (1.0%) and KNO_3 (2.0%) recorded 32.7%, 26.7% and 27.5% higher seed yield than control, respectively. Foliar application of sodium benzoate was least effective in increasing seed yield in our study but both the concentrations recorded significantly more seed yield as compared to control. The increase in seed yield with foliar application of above mentioned bio-regulators was due to increase pod length, pods plant^{-1}, seeds pod^{-1}, seed yield plant^{-1} and 100-seed weight (Table 1). Salicylic acid at lower concentration stimulate photosynthetic machinery, improves chlorophyll and protein synthesis, inhibit ethylene biosynthesis and increase seed and stover yield through reducing adverse effects of heat stress in many field crops (Wahid *et al.* 2007; Kumar *et al.* 2013).

Salicylic acid protects cell membranes and their binding transporter proteins which maintain their structure and function against the toxic and destructive effects of reactive oxygen species (ROS) released during heat stress (Hayat *et al.* 2009; Hayat *et al.* 2010; Kumar *et al.* 2013; Bons *et al.* 2015). Foliar spray of lower concentrations of salicylic acid conferred heat tolerance in plants due to enhanced H_2O_2 level and reduced the catalase (CAT) activity, thereby increasing the potential of plants to withstand the heat stress (Hayat *et al.* 2010). The exogenous application of salicylic acid has been reported to increase seed and stover yield through reducing adverse effects of heat stress on biochemical changes in many field crops (Wahid *et al.* 2007; Kumar *et al.* 2013; Bons *et al.* 2015). The increase in stover yield observed in our study was due to increase in plant height and weight plant^{-1} (Table 1). These favorable changes with the application of salicylic acid along with observed increase in chlorophyll content of leaves and plant height in our study possibly enhanced photosynthetic efficiency due to increased SPAD value resulting in

Table 3. Effect of year on crop parameters and seed yield of cowpea (data averaged across the cultivars and foliar treatments)

Year	SPAD Value	Plant height (cm)	Fresh weight $plant^{-1}$ at harvest	Pods $plant^{-1}$	Pod length (cm)	Seeds pod^{-1}	Seed yield $plant^{-1}$	100-seed wt. (g)	Total seed yield (t ha^{-1})	Stover yield (t ha^{-1})
2008	50.1a	198.1a	201.0a	25.1a	15.3a	15.4a	33.6a	13.1a	1.44a	9.01a
2009	47.2b	185.0b	185.0b	22.1b	13.8b	12.6b	30.5b	11.2b	1.35b	8.59b

Values followed by same lower-case letters within a column are not significantly different at $p = 0.05$.

greater food reserve for the formation of seed thereby increasing number of seeds pod^{-1}, 100-seed weight, pod length and seed yield in cowpea.

In an earlier study, Murtaza *et al.* (2007) recorded significant increase in 100-seed weight and seed yield of pea (*Pisum sativum*) with seed and foliar treatment of salicylic acid (10^{-4} *M*) as compared to control. The decrease in seed yield with high dose of salicylic acid (100 µg mL^{-1}) might be due to its detrimental effect on photosynthetic activity and inhibition of nitrate uptake system as reported by Kumar *et al.* (2013).

K availability from KNO_3 promotes photosynthesis, enhance water uptake and root permeability and thus improve the seed yield attributes and its quality under heat stress conditions (Kumar *et al.* 2013). Foliar sprays of 0.5% and 1.0% $CaCl_2$ increased seed yield of cowpea by 19.8% and 23.5%, respectively over control. Calcium (Ca^{+2}) availability from $CaCl_2$ regulates many biochemical processes and play important role in cell function and signal transduction, delaying senescence, decreasing flower abscission in crops due to heat induced oxidative damage (Wahid *et al.* 2007) and thus improve the seed yield of crops. Calcium application in the form of $CaCl_2$ prior to heat stress increases the malondialdehyde content which stimulate the activities of guaiacol peroxidase, superoxide dismutase (SOD) and catalase (CAT), which could be reasons for the induction of heat tolerance (Waraich *et al.* 2012). Increase in number of pod sets and seed yield with foliar application of Ca at flowering and pod setting stage in *Phaselous vulgaris* and *Egyptian clover* has also been reported by El-Abou (2011) and Kumar *et al.* (2013). Stover yield of cowpea also followed the same trend as seed yield. Harvest index was not significantly affected by application of bio-regulators (Table 2).

Effect of on seed quality

The study revealed a different response of two cultivars of cowpea seed produced under foliar application of different bio-regulators. Cultivars CL 367 and Cowpea 88 did not differ significantly with respect to seed germination (Table 2). However, shoot and root length and seedling vigor index were found to be significantly higher in cultivar CL 367 indicating that it is a better cultivar than Cowpea 88 which might be possibly due to its genetic makeup (El Naim and Jabereldar, 2010).

All the bio-regulators showed significant effect on the different traits of seed quality (Table 2). Averaged over two years, foliar application of salicylic acid at 50 µg mL^{-1} resulted in maximum increase (14.1%) in seed germination, shoot length (17.6%), root length (23.1%) and seedling vigor index (36.5%) closely followed by 2.0% KNO_3 and 1.0% $CaCl_2$ over control (Table 2). Sodium benzoate was least effective in improving the seed germination and seedling vigor index. The protective and growth promoting effects of salicylic acid at 50 µg mL^{-1} are presumably due to bold seeds, increased level of cell division within the apical meristem of seedling root and is reported to enhance root and shoot growth in soybean and other crops through the regulation of photosynthetic reactions (Wahid *et al.* 2007; Kumar *et al.* 2013). The significantly greater radicle elongation under foliar application of salicylic acid observed may have interesting implications because it may allow the root to exploit the water reserves in the deepest layer of soil under drought and water stress condition arisen due to higher temperature. Similarly seeds produced under KNO_3 and $CaCl_2$ foliar sprays had higher food reserves due to more photosynthesis and might be reason for better seed quality traits than control in our study. Higher concentration of salicylic acid (100 µg mL^{-1}) significantly reduced the root length and seedling vigor index compared with 50 µg mL^{-1}.

Year's effect

At harvest significantly higher SPAD values, plant height and weight $plant^{-1}$ was recorded in 2008 than in 2009 (Table 3). Yield attributing parameters such as pods $plant^{-1}$, pod length, seeds pod^{-1}, seed yield $plant^{-1}$ and 100-seed weight were significantly more in 2008 than in 2009.

Similarly, both seed and stover yields were also significantly lower in the 2009 compared with that in 2008. Higher growth and yield attributing parameters in cowpea during the year 2008 were possibly due to higher rainfall and lower minimum temperatures during reproductive phase (20.9°C and 21.9°C) compared to that in 2009 (24.3°C and 24.7°C), which might had favored the greater transfer of nutrients and photo assimilates due to enhanced photosynthetic activity (higher SPAD chlorophyll value) during growth as well as reproductive stages of cowpea. Consistent with our study, Babaji *et al.* (2011) also reported better performance of cowpea in terms of growth parameters and seed yield in the year with higher rainfall and milder temperatures than in the other year of study. Decreases in seed yield of cowpea due to excessive floral abscission, lower pod set, reduced photosynthesis, and lower seed weight at high night temperatures (>20°C) has been reviewed by Wahid *et al.* (2007). Harvest index and seed quality traits (germination percent, root length, shoot length and seedling vigor index) were similar in both the years of study (data not reported).

Conclusion

From this study it is concluded that, cultivar CL 367 performed better in terms of seed and stover yield than Cowpea 88. Among the various bio-regulators, three foliar applications of salicylic acid (50 µg mL^{-1}) followed by 2.0% KNO_3 and 1.0% calcium chloride, proved very effective in increasing yield attributing characters and seed yield. Sodium benzoate did have least positive effect on cowpea seed yield. Seed quality traits like germination percentage, shoot length, root length and seedling vigor were also markedly improved with the foliar spray of 50 µg mL^{-1} salicylic acid. Considering the amount and cost of the bio-regulators required for the foliar sprays, salicylic acid is more economical. The increase in seed yield will further boost area under this important legume crop in tropical and subtropical areas thereby sustaining the livestock productivity.

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