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Identification of seedling root morphological traits associated with economic yield and yield components of cowpea (*Vigna unguiculate*L.)

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ABSTRACT

Cowpea (Vigna unguiculata L.*) is well adapted to relatively dry and less fertile environments hence root system characteristics are very important for water and nutrient acquisition. The present study was conducted to identify the relationship between economic yield and yield components with seedling root traits of recommended cowpea varieties in Sri Lanka. Three experiments under controlled environment conditions were conducted at Faculty of Agriculture, University of Ruhuna. Eight cowpea varieties (Var. Dawala, Waruni, Vijaya, Bombay, MI35, MICP1, ANKCP1 and ANKCP2) were used. A pot experiment was conducted in protected house and set up according to randomized complete block design with three replicates while two lab experiments were arranged to evaluate seedling root traits by using completely randomized design with five replicates. Number of branches per plant, number of pods per plant, average pod length, number of seeds per plant, 100 seed dry weight, total number of active nodules per plant and root dry weight at maturity were measured. Length of the taproot, total root length, number of lateral roots per plant and root dry weight was recorded in seedlings. All yield parameters and root traits measured were significantly different among cowpea varieties (P < 0.001). Seedling taproot length and number of lateral roots per plant varied significantly with the growth condition where var. Waruni and Bombay recorded the highest tap root length in sand media and var. Dawala and ANKCP2 in wet cotton wool media.Number of lateral roots per plant was always high in wet cotton wool media when compared to sand media. A great number of active nodules per plant were observed in var. Bombay and ANKCP2. Seedling root traits were positively correlated with the yield components namely average pod length, number of seeds per pod and 100 seeds dry weight.*

Key words: *Cowpea, Economic yield, Root morphology, Seedlings, Yield components*

INTRODUCTION

Legumes is one of the most ancient food crops known to man and cultivated for grains, green pods and fodder (Jeswani and Baldev, 1990). Farmers use legumes in different cropping systems and farming methods, even before the discovery of its ability to fix atmospheric nitrogen. Cowpea (*Vigna unguiculata* L.) is an important legume crop can be cultivated throughout the year in most of the tropical countries. It is one of the major income generation crops which contributes massively on food security of the people in tropical and subtropical countries (Adu, *et al*., 2019; Tharanathan and Mahadevamma, 2003). Cowpea is well adapted to relatively dry and less fertile soils and mainly cultivated in Asia, Africa, Southern Europe and Central and South America.

Cowpea is one of the major cultivated legume crops in Sri Lanka.It is mainly cultivated in Hambanthota, Rathnapura, Badulla, Monaragala, Anuradhapura, Kurunagala, Puttalam, Batticaloa, Ampara districts in Sri Lanka. Legumes have significant nutritional as well as economic importance (Senaratne *et al*., 1998). However, pulse production in Sri Lanka is insufficient to meet national needs. Over Rs.2 billion are spent annually to import

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legumes, mainly of lentil and chickpea. Cowpea, mung bean, black gram, and groundnut are the major growing legumes in Sri Lanka, on about 80,000 ha. Average annual production of cowpea in 2018 was 1.18 t/ha (AgStat, 2019). Though there are few recommended varieties produced by the DOA Sri Lanka, the popularity of them among the farmers' are at the considerably low level (Hewavitharana *et al*., 2010; Millawithanachchi *et al*., 2012). There were about eight recommended cowpea varieties in Sri Lanka; Dawala, Waruni, Vijaya, Bombay, MI35, MICP1, ANKCP1 and ANKCP2.

Cowpea production of Sri Lanka varies from year to year (Department of Census and Statistics, 2018). One of the main objectives of the cowpea breeders in Sri Lanka is to enhance the economic yield of the crop. However, attention on root morphological characteristics and their relationships with economic yield were not given. Most plant breeding efforts fail to focus on the relationship between the root system and crop yield, probably because of the underground location of the root system. However, there could be a good relationship between root traits and yield of the crop including cowpea. Further, the interaction between root traits in the seedling stage and the final yield could be identified in cowpea. The plant breeding attempts should be centered on the modification of root traits to increase crop yield. There is a possibility to develop stress-tolerant crops and enhance the capacity of the plant to explore the soil for better absorption of water and nutrients (Paez-Gracia *et al*., 2015). The root system of the plant is the interface between plant and soil nutrients. Therefore, roots actively participate to obtain nutrient from soil and help to achieve the yield in plants (Akpan and Mbah, 2016). Determination of the morphological and spatial distribution of the root system of the plant is important to quantify the availability of water and nutrients to the plant and it helps for improving crops and managing soils to obtain high crop yield (Gregory, 2009). Therefore, the present study was conducted to find out the relationship between seedling root traits and economic yield and yield components of cowpea. Specific objectives of the study were to compare cowpea varieties for yield component and economic yield and to study the phenotypical variation of seedling root systems of cowpea varieties under different growth conditions

MATERIALS AND METHODS

Experimental site

Three control environment experiments were conducted at Faculty of Agriculture, University of Ruhuna, Sri Lanka from October, 2019 to February 2020, to identify the seedling root morphological traits associated with economic yield and yield components of cowpea.

Experiment 1

Experimental design and treatments

The first experiment was conducted in pots to evaluate the economic yield and yield components of eight cowpea varieties. A randomized complete block design was used in this experiment with three replicates and eight treatments. Treatments for all experiments were cowpea varieties of Dawala, Waruni, Vijaya, Bombay, MI35, MICP1, ANKCP1 and ANKCP2. The potting media used for the experiment was top soil: sand: compost into 2:1:1 ratio. Both basal and top dressings were done according to the recommendations of Department of Agriculture, Sri Lanka. The basal dressing was applied for the potting media, one day before the seed sowing and top dressing was applied for the plants at the flowering stage (on set of flowering). Seeds were soaked in water for 24 hours and kept in dark for another 24 hours to synchronize the germination. After that, germinated seeds were placed in the pots, randomly within the block (three seeds per pot). After two week of germination, two seedlings were removed from the pot by leaving a single plant per pot. Irrigation was done at immediately after seed sowing and thereafter as needed. Normally in vegetative stage, irrigation was done with two days interval and during flowering and pod bearing stage irrigation was done in every day. Hand weeding was practiced to control weeds. First weeding was done at 15 days after seed sowing and then weeding was done when necessary. First pinching was done at 20 days after seed sowing and second pinching was done when axillary buds developed shoots. This was done to form bushy appearance of the plant hence, get more productive branches. All the agronomic practices were done according to the recommendation of Department of Agriculture, Sri Lanka.

Plate 1: Morphological differences between seeds of cowpea varieties used in the study

Cowpea pods were harvested when they attain the physiological maturity stage. Pods were harvested when they turn light straw in color and as picking. Harvesting was started about 50-60 days after seed sowing and harvesting was continued with 5-6 days interval. Seeds were separated from pods manually and sun-dried.

Measurements

Number of branches per plant, number of pods per plant, average pod length, number of seeds per pod and 100 seeds dry weight wererecorded. The number of active root nodules and root dry weight were taken at harvest.

Number of branches per plant

The number of branches from each plant was counted and finally, the average number of branches per plant of each treatment was taken from three replicates.

Number of pods per plant

The number of pods per plant was counted in each harvesting session. Finally, the average number of pods per plant was taken for each treatment from three replicates.

Average pod length

After harvesting of pods, five pods from each plant were selected randomly and measured the average pod length per plant. Pod length was taken as an exterior distance from the peduncle connection point to the apex excluding beak of the pod using a ruler.

Number of seeds per pod

Ten pods from each plant wereselected randomly and count the number of seeds per pod, then get average to calculate the number of seeds per pod in each plant.

100 seed dry weight

After getting the total yield of the plant, randomly select 100 seeds from each treatment and measure the weight of the sample.

Root dry weight

At final harvest, plants were uprooted and washed out the soil carefully. Then roots were put into labelled paper bags and oven-dried at 65^0C for 72 hours until getting the constant weight. Finally, dry weight was recorded. Average of three replicates in each treatment was recorded as the final root dry weight.

Total number of active nodule per plant

After uprooting of the plants, the root system was separated from shoots. These roots gently washed under a tap over a fine sieve to remove all soil particles and dust. Then the nodules were detached manually from the roots and counted. Only fresh and active nodules were considered. All the nodules were cut and opened to recognize them as fresh and active. Active nodules have pink color pigments inside.Finally the average number of nodules per plant was measured by three replicates.

Experiment 2 and 3

Experimental design and treatments

Second and third experiments were arranged according to completely randomized design with eight cowpea varieties and five replicatesto evaluate seedling root morphological traits. The media used in the second experiment was wet cotton wool while in the third experiment was sand.

Preparation of growing media

In the second experiment, forty petri dishes were cleaned and cotton wool was spread in the petri dishes. Then water was sprayed to wet the cotton wool. After that, wet tissue papers were laid on the wet cotton wool and seeds were placed on wet tissue paper. Finally, all these petri dishes were covered by black polythene. Water was sprayed to the petri dishes in two days intervals to maintain the moisture content of the petri dishes.

In the third experiment, the glass boxes (20 cm x 20 cm x 1 cm) filled with washed fine sands were used as a media for the experiment. After that, germinated seeds were placed in sand media of the box close to the edge. Then the roots were developed in sand media near to the edge of the boxes. Watering was done to maintain the wet condition in sand media.

Measurements

Length of taproot

The length of the taproot was measured from the point where the stem and root system separated to the tip of the main root.

Total root length

The length of each lateral root was measured and got the total lateral root length for each seedling. Finally, the sum of the lateral roots length and the taproot length is called total root length.

Number of lateral roots per plant

The roots which emerged from the taproot were counted as lateral roots.

Seedling root dry weight

100 seeds from each variety were selected to get seedling root dry weight. These seedswere kept in the same condition of experiment 2; on the wet cotton wool. After ten days, the root system was removed from the shoot and oven-dried in 65^0C for 72 hours to obtained constant weight.

Data analysis

Data analysis was done using SAS, Minitab 17 and MS excelsoftware. Pearson correlation was used to find out the relationships between each variable using Minitab 17 software and mean separation was done using DMRT. Descriptive data were presented using graphs.

RESULTS AND DISCUSSION

Yield parameters

The results of different yield components of cowpea varieties are presented in Table 1. The number of branches per plant, number of pods per plant and average pod length was significantly different (P<0.05) among treatments. However, the average number of seeds per pod was not significantly different among treatments.It may be due to the fact that the number of seeds per pod depend on pod length and size of the seeds (Siemens and Johnson, 1995).

Table 1: Number of branches, number of pods per plant, average pod length and number of seeds per pod of eight cowpea varieties

Treatment (Variety)	Number of branches	Number of pods per plant	Average pod length (cm)	Number of seeds per pod
Dawala	9.33^{a}	18.33^{a}	15.00^{a}	13.00^{a}
Waruni	7.33^{bc}	12.00^{cd}	12.80^{bc}	11.33^{ab}
Vijaya	5.67^{d}	15.00^{abc}	12.43^{bc}	11.67^{ab}
Bombay	7.00^{cd}	10.33^{d}	13.43^{b}	12.33^{ab}
MI35	8.33^{ab}	14.00^{bc}	10.17^{d}	12.33^{ab}
MICP1	6.33^{cd}	15.67^{ab}	11.93^{c}	12.33^{ab}
ANKCP1	5.67^{d}	15.33^{ab}	13.20^{bc}	10.67^{b}
ANKCP2	9.00^{a}	15.67^{ab}	16.10^{a}	13.33^{a}
P value	<0.001	<0.001	< 0.0001	0.204
CV%	5.735	5.951	5.445	4.673

Remarks: Values in each column followed by the same letter are not significantly different at 5% probability level according to DMRT. CV%- Coefficient of variance

The highest number of branches per plant was observed in var. Dawala, but it was not significantly different from var. MI35 and var. ANKCP2. The lowest number of branches per plant was not significantly different invar. Vijaya and var. ANKCP1. According to Muhaijeer *et al* (2008), the highest number of branches was observed in var. MI35 and lowest numbers of branches were observed in var. Waruni when compared to Dawala, waruni and MI 35 cowpea varieties in Batticaloa district.

The number of branches per plant directly related to the number of flowers produced in the plant hence contributed to the economic yield (Abearathne *et al*., 2013).

The average yield of each cowpea variety was calculated. The highest average yield per plant was recorded in var. ANKCP2 but it was not significantly different from var. Dawala (Figure 1). Var. MI35 recorded the significantly lowest average yield per plant, though it was not significantly different from var. Waruni. According to Abearathne *et al* (2013), among four cowpea varieties (Dawala, Waruni, Bombay, MI35) recommended by DOA, var.MI35 has the most attractive cream-colored seed coat but has low yield when compared with other varieties. Therefore, Field Crops Research and Development Institute (FCRDI) successfully conducted a back cross-breeding program and introduced high yielding var. MICP1 with seeds coat color of var. MI35.Var.MICP1 performed well and recorded high yield than var. MI35.

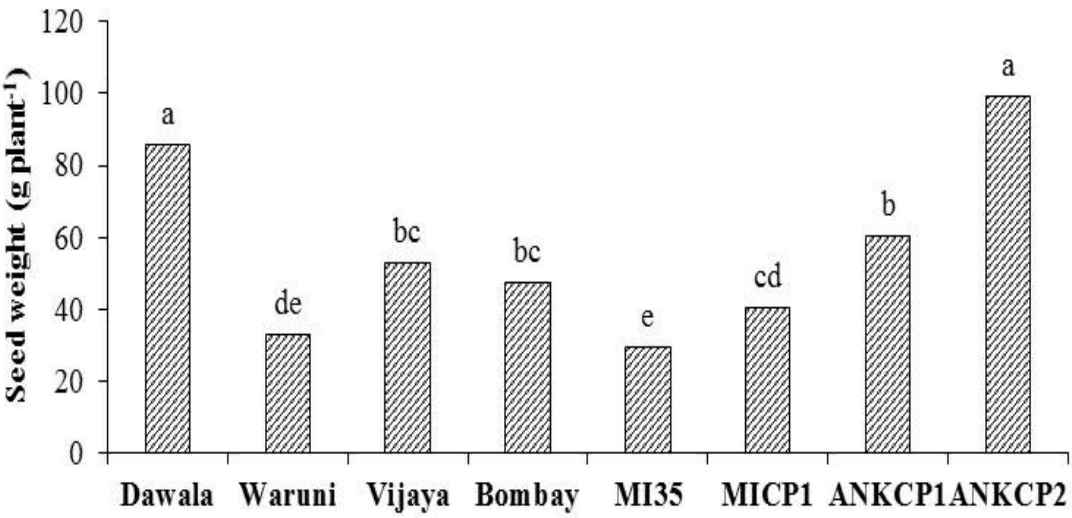


Figure 1: Average yield per plant of eight cowpea varieties ($P < 0.001$)
Remarks: Means with the same letter are not significantly different at 0.05 levels

The highest average pod length was observed in var. ANKCP2 and not significantly different from var. Dawala. The lowest average pod length was observed in var. MI35 and it showed a significant difference with other varieties.
The highest 100 seeds dry weight was recorded in var. ANKCP2 (47.75g) and the lowest 100 seeds dry weight was shown in var. MI35 (17.00g). Size of the seeds affects 100 seeds dry weight.Var. MI35 had the smallest size of seeds and var. ANKCP 2 had the largest size of seeds among these eight varieties. Similar results were reported by Ali *et al* (2013) where bean varieties with larger pods gave larger seeds leading to higher weight for a set number of grains. Msolla and Mduruma (2008) reported that the weight of 100 seeds for a given variety may be related to the time taken to maturity implying that early maturing varieties could have heavier seeds.

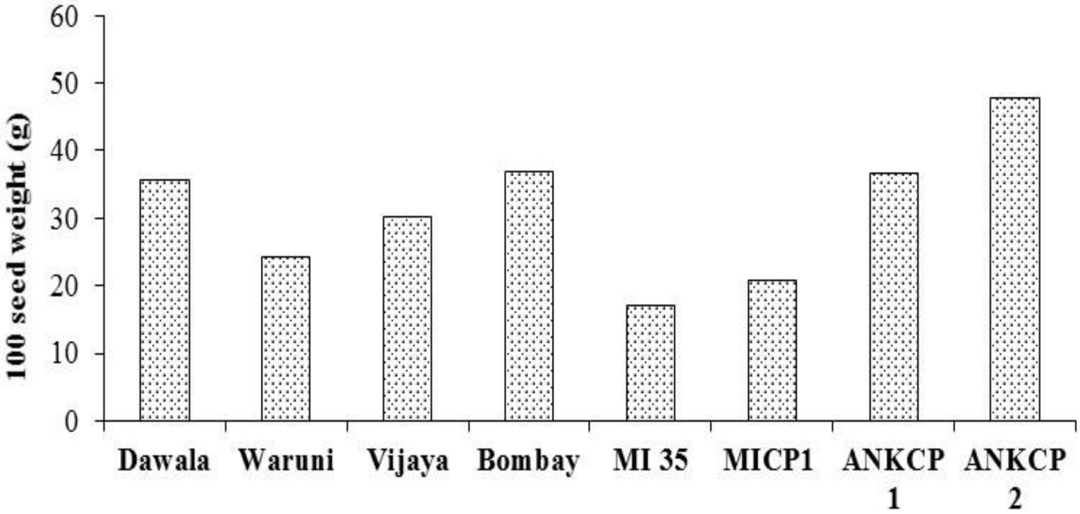


Figure 2 : 100 seeds dry weight of eight cowpea varieties ($P< 0.001$)

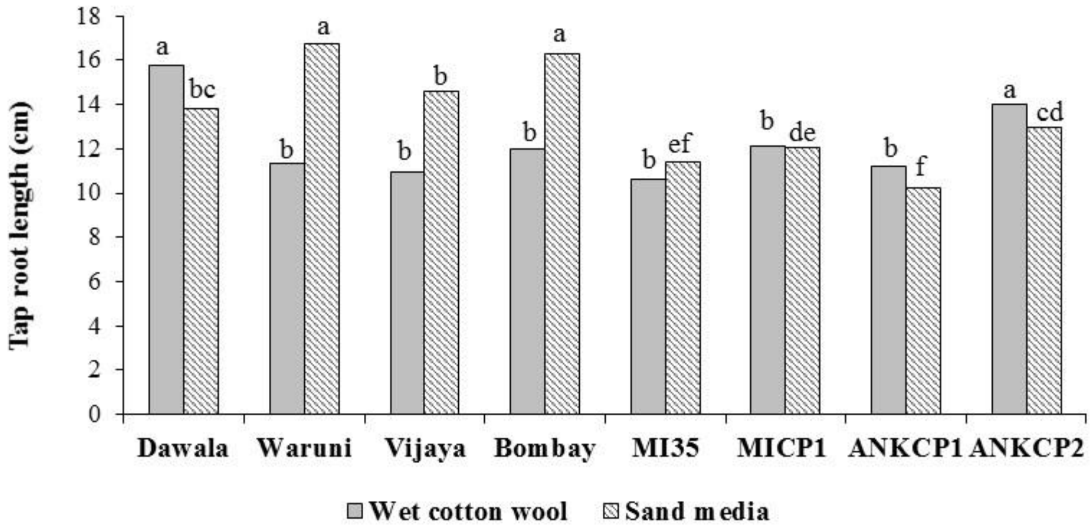
Root morphological traits

Table 2 shows the root morphological traits of cowpea varieties in three experiments. Root system traits were evaluated in different growth stages of plants (maturity and seedling stage) and under different growing media for seedlings (wet cotton wool and sand).
Root dry weight at maturity was significantly different ($P<0.001$) among cowpea varieties. The highest root dry weight was recorded in Var. MICP1 while the lowest value was recorded in var. MI35. The lowest root dry weight in the seedling stage was also showed in var. MI35. However, var. Dawala recorded the highest root seedling dry weight though it was not significantly different from var. Bombay.

In the seedling stage, taproot length was significantly different ($P< 0.001$) among treatments for both growing media. When seeds sown on wet cotton wool, numerically highest tap root length were shown in var. Dawala but it was not significantly different from var. ANKCP2. The lowest tap root length was recorded in var. MI35 but it was not significantly different from var. Waruni, var. Vijaya, var. Bombay, var. MICP1 and var. ANKCP1. When seeds were sown in sand media, var. Waruni recorded the longest taproot length though it was not significantly different from var. Bombay. The significantly lowest tap root length was observed in var. ANKCP1 (Figure 3).

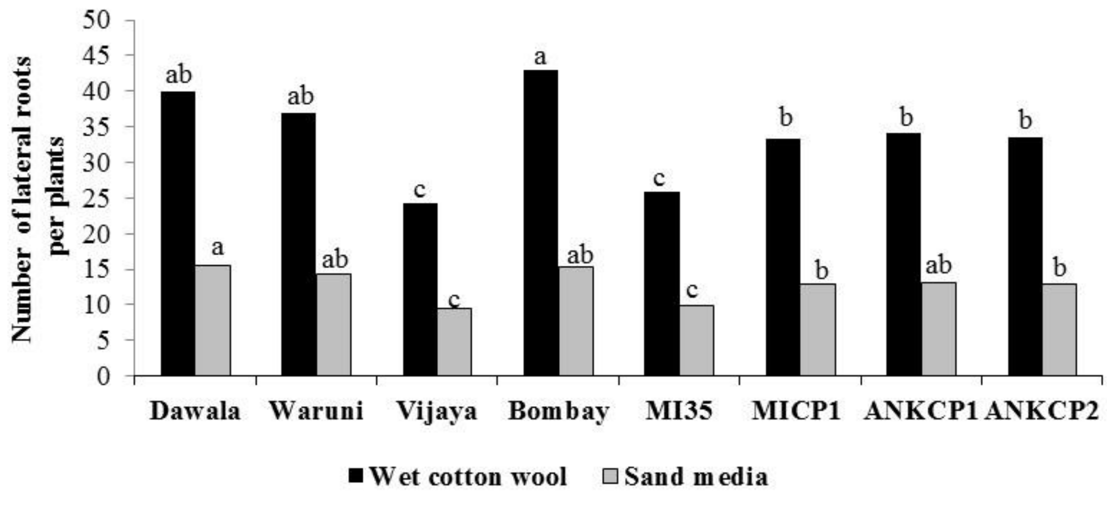
The number of lateral roots in seedling root systems was significantly different among varieties ($P<0.001$) in both media. When seeds were sown on wet cotton wool, the highest number of lateral roots per plant was recorded in var. Bombay. The lowest number of lateral roots per plant was recorded invar. Vijaya and it was not significantly different from var. MI35. When seeds were sown in sand media, var. Dawala showed the highest number of lateral roots but it did not significantly different from var. Waruni, var. Bombay and var. ANKCP1. The lowest number of lateral roots was recorded in var. Vijaya. However, it was not significantly different from var. MI35 (Figure 4). The longest total root length was obtained in var. Dawala and var. Bombay while the lowest values were recorded in var. Vijaya and var. MI35.

Plants can customize the root architecture to adapt to different environmental conditions, like stress, by integrating with the genetic programs to control root growth (Jovanovic *et al.*, 2008). The root structure of the plant, number and distribution of root architecture, is different from plant to plant because of the genetic and nutritional conditions of the plants. It allows to plant to recover in stress conditions (López-Bucio *et al*., 2003).



Means with the same letter are not significantly different within the growing media at 0.05 probability level

Figure 3: Taproot length of seedlings in wet cotton wool media and sand media in second and third experiments, respectively ($P < 0.001$)



(Means with the same letter are not significantly different within the growing media at 0.05 levels)

Figure 4: Number of lateral roots per plantin wet cotton wool media and sand media in second and third experiments, respectively ($P < 0.001$)

Table 2- Root morphological traits of cowpea varieties in three experiments

Treatment (Varieties)	Experiment 01 (at maturity)	Experiment 02 (Seedlings in wet cotton wool)				Experiment 03 (Seedlings in sand)	
	Root dry weight (g)	**Seedling tap root length (cm)**	**Number of lateral roots**	**Total root length (cm)**	**Seedling root dry weight (mg)**	**Seedling tap root length (cm)**	**Number of lateral roots**
Dawala	0.42^{d}	15.76^{a}	40^{ab}	55.76^{a}	12.8^{a}	13.8^{bc}	15.6^{a}
Waruni	0.48^{d}	11.36^{b}	37^{ab}	48.36^{ab}	8.8^{d}	16.78^{a}	14.4^{ab}
Vijaya	0.56^{cd}	10.96^{b}	24^{c}	35.16^{c}	9.3^{d}	14.60^{b}	9.4^{c}
Bombay	1.19^{bc}	11.96^{b}	43^{a}	54.96^{a}	12.0^{b}	16.26^{a}	15.4^{ab}
MI35	0.25^{d}	10.60^{b}	26^{c}	36.40^{c}	7.8^{e}	11.40^{ef}	9.8^{c}
MICP 1	1.91^{a}	12.14^{b}	33^{b}	45.54^{b}	10.0^{c}	12.04^{de}	12.8^{b}
ANKCP 1	0.73^{cd}	11.18^{b}	34^{b}	45.38^{b}	10.3^{c}	10.20^{f}	13.2^{ab}
ANKCP 2	1.25^{b}	14.04^{a}	34^{b}	47.64^{ab}	11.7^{b}	12.98^{cd}	12.8^{b}
P value	<0.001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CV%	41.92	11.79	7.45	6.38	2.90	7.55	14.73

Remarks: Values in each column followed by the same letter are not significantly different at 5% probability level according to DMRT. CV%- Coefficient of variance

The number of active nodules per plant is one of the important parameters to determine the effectiveness of biological nitrogen fixation process. Nodules can be visible in plant roots and can be observed by the naked eye. In the young stage, these nodules do not fix nitrogen and they are white or grey color inside. When they become mature, they turn to pink or reddish in color, and then the N fixation is started. These pink or reddish colors of nodules occur due to leghemoglobin (Franko- Lindberg and Dahlin, 2013). Number of active nodules per plant is significant (*P*<0.001) among cowpea varieties. The comparatively higher number of nodules per plant was obtained in var. ANKCP2 and lowest number of nodules per plant was recorded in var. MI35 (Table 3).

Table 3- Number of nodules in cowpea varieties

Treatments	**Number of active nodules per plant**
Dawala	61.67^{bc}
Waruni	66.33^{bc}
Vijaya	61.67^{bc}
Bombay	78.67^{ab}
MI35	33.33^{d}
MICP1	59.33^{c}
ANKCP1	74.67^{bc}
ANKCP2	91.00^{a}
P value	<0.001
CV%	7.279

Remarks: Values in each column followed by the same letter are not significantly different at 5% probability level according to DMRT. CV%- Coefficient of variance

Relationship between root traits and yield components

Correlation matrix between yield components and root system architecture is given in Table 4. In the present study, number of active nodules per plant and average pod length showed a positive correlation (*P*<0.05) (r = 0.825). When increasing the number of active nodules per plant average pod length was also increased in cowpea. Further, the number of active nodules per plant was significantly correlated (*P*< 0.05) with the number of pods per plant and 100 seeds dry weight at maturity (Table 4). According to Ittah and Arua (2017), root density was moderately correlated with the number of pods per plant but poorly with the number of seeds per pod. A negative moderate correlation was observed between root volume and number of pods per plant. However, there was a poor correlation between 100-seeds weight and root volume. Taproot length of the seedling grown on wet cotton wool had a positive correlation between average pod length (*P*< 0.05, r = 0.79) and the number of seeds per pod (*P* < 0.05, r = 0.71) at maturity. Further, root dry weight of seedlings correlated (P <0.05) with average pod length (*P*< 0.05, r = 0.84) and 100 seeds dry weight (*P*<0.05, r = 0.77).

Most research studies on crop yield improvement focus on the traits associated with shoot biomass production, branching ability, diseases and pests resistance while hardly on the root traits. The root system is underground in most plant species; it is a very important character in plants because of its roles in water and nutrient absorption from the soil, in anchorage and interfacing with the soil microorganisms. According to Akpan and Mbah (2016), the root system is the link between soil nutrient and plant productivity.

Table 4– Correlation coefficient and *P* values between root traits and yield components

Plant stage	Parameters	Number of branches	Number of pods/plant	Average pod length	Number of seeds/pod	100 seeds dry weight
at maturity	Root dry weight	-0.230* 0.584**	-0.040 0.924	0.184 0.663	0.256 0.541	0.159 0.707
	Number of active nodules/plant	-0.026 0.951	0.094 0.025	0.825 0.012	0.096 0.821	0.874 0.005
Seedlings(wet cotton wool)	Tap root length	0.702 0.052	0.596 0.119	0.794 0.019	0.709 0.049	0.557 0.152
	Number of lateral roots	0.259 0.536	-0.215 0.609	0.532 0.175	0.169 0.689	0.406 0.319
	Total root length	0.387 0.343	-0.044 0.918	0.644 0.085	0.312 0.451	0.479 0.229
	Root dry weight	0.361 0.380	0.280 0.502	0.835 0.010	0.505 0.202	0.774 0.024
Seedlings (sand media)	Tap root length	0.079 0.852	-0.554 0.154	0.195 0.644	0.084 0.844	0.071 0.867
	Number of lateral roots	0.288 0.489	-0.100 0.813	0.567 0.143	0.149 0.729	0.393 0.335

Each cell contain Pearson correlation coefficient* and *p*- value**, correlation is significant at 0.05% probability level;

Omondi (2013) reported that genetic variability affects to determine the performance of bean. Dahmardeh *et al* (2010) documented that varietal difference influencing yield characteristics for varieties grown under similar conditions. Male-Kayiwa (2000) agrees that edaphic factors do influence the productivity of the bean crop besides the genetic make-up as does Shenkalwa (2013) found that yield is a factor of both genotype and environmental factors.

Some root traits such as root volume, root biomass and root length are controlled by multiple genes, indicating that non-genetic factors would play a significant role in the inheritance of the traits, whereas the yield traits such as the number of seeds per plant, pod length and number of pods per plant are controlled by single genes (Sangwan and Lodhi, 1995). Cowpea is a self-pollinated crop, therefore adoption of mass selection as a breeding method to improve root and yield traits at the same time would be slow. Shenkalwa *et al* (2013) reported in their work when examining the performance of improved bean varieties reported that improving the characteristics of old varieties improved their performance when compared to performance in the past.

CONCLUSION

According to the results of the three experiments, it can be concluded that all of the yield parameters and root traits measured in the experiments were significantly different among cowpea varieties. Seedling taproot length and number of lateral roots per plant varied significantly with the growth condition. Seedling root traits were positively correlated with some yield components such as average pod length, number of seeds per pod and 100 seeds dry weight.

Root morphological traits have not been considered when breading cowpea for high yield. However, there are positive andnegative relationships were identified between early seedling root traits and yield components. Therefore, when studying breeding populations, it is worth looking into root morphological traits associated with yield components of cowpea in the future.

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