EFFECT OF NAPHTHALENE ACETIC ACID, STAKING, NITROGEN AND PHOSPHORUS APPLICATION ON SEED QUALITY OF THE CLIMBING BEAN (*Phaseolus vulgaris L.*)

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Abstract

Seed quality plays an important role in seedling emergence, plant stand establishment and yield potential. Therefore, the use of good quality seeds is essential to obtain an optimum plant population, growth vigour, and optimal yield per unit area. Conditions prevailing during seed formation can affect the quality of seed produced and ultimately crop yield. Agronomic practices may affect the seed quality. Therefore, there is need to test the effect of different agronomic practices on quality of seed. Field experiments were conducted at Kaguru Farmers Training Centre, Kenya, to determine the effect of Naphthalene Acetic Acid (NAA), staking, and application of nitrogen (N) and phosphorus (P) on seed quality of the climbing bean. The experiment was laid out in Randomised Complete Block Design, with three factors, i.e. NAA concentrations (0, 0.2 and 0.4 mlL^{-1}), staking (no staking, staking with stakes and maize plants as support), and Diammonium Phosphate (DAP) [0, 200, 250 and 300 Kg DAPha^{-1}] which supplied N and P. After harvesting the climbing bean laboratory analysis was done and data on seed nitrogen, phosphorus and protein content determined. The data collected was subjected to analysis of variance using SAS version 9.4 and significant means separated using LSD at $\alpha= 0.05$. The finding of the study revealed a significant ($p < 0.05$) treatment effect on phosphorus and protein content of the seeds of climbing bean. The application of 0.4 mlL^{-1} NAA and 300 Kg DAPha^{-1}, and staking with stakes resulted in the highest phosphorus (0.6240 %) content of the harvested seed. The application of 0.4 mlL^{-1} NAA and 300 Kg DAPha^{-1}, and use of maize plants as support resulted in the highest seed protein content (30.54 %) of the climbing bean. The findings of the study demonstrated that application of NAA, staking with stakes, N and P had an apparent effect on seed P, N and crude protein.

Keywords: Climbing beans, Naphthalene acetic acid, Seed nitrogen content, Seed phosphorus content, Seed crude protein content, Staking.

INTRODUCTION

Seed quality is one of the most important factor in crop production, human nutrition and food security (Finch–Savage and Bassel, 2016). Seed quality is a result of genotype and the growing environment of mother plant (Moussavi, 1997). The seed tissue mineral reserves that are dependent on supply of nutrients to the mother plant may determine plant vigor and performance of resultant plants. Hasan *et al.* (2019) indicated that among several agronomic and management practices, the mother plant nutrition plays an important role for increasing production of quality seed. Successful seedling establishment is the first critical step in crop production that determines success or failure of future harvest (Finch – Savage and Bassel, 2016). In a production system, farmers should use high quality seeds to obtain the right plant population per unit land area for maximum production. Seed viability and vigour is a major determinant of successfully seedlings emergence, plant stand establishment and ultimate yield of a given crop (Rajala *et al*, 2011). These two traits are affected by several genetic and agronomic factors, starting from planting throughout harvest and storage conditions.

Agronomically, seed quality can be improved by managing seed crops to aid accumulation of mineral nutrient in the seeds followed by grading to select only the large seeds for sowing (Moussavi, 1997). An imbalance of nutrients in the soil or an excess of ions may affect seedling development through an initial effect on the seed growth (Martínez-Ballesta *et al.,* 2020). The quality of the seed determines the emergence, vigour and the growth of seedlings. Agronomic practices such as application of fertilisers, staking and phytohormones can affect the seed quality of climbing bean plants. Plant hormones play important roles in controlling how plants grow and develop and they have been implicated in a wide range of physiological functions including regulation of plant architecture, photomorphogenesis, seed germination, nodulation, and physiological reactions to abiotic factors (Faizan *et al.,* 2020). Application of phytohormone have been shown to affect seed germination and seedling vigour (Ojo *et al*., 2017). Naphthalene acetic acid (NAA), a synthetic plant hormone, has been found to influence plant nutrient uptake and protein content of the seed (Abd *et al*., 2018). Abd *et al*. (2018) reported that application of NAA improved uptake of nitrogen (N), phosphorus (P), potassium (P), calcium (Ca) and magnesium (Mg) in cucumber crop. Moreover, Karim, *et al.,* (2003) reported that higher protein content of 23.99 % was obtained with the application of NAA in soyabean seeds. Auxins has been found to stimulate protein synthesis in soya bean (Nascimento and Mosquim, 2004).

Staking is a training practice in climbing crops, and involves providing support to climbing crops such as climbing beans due to their indeterminate growth habit (Lwakuba, 2003). Staking enhances greater vegetative growth that is crucial towards supporting the reproductive phase. On the other hand, the application of N and P increases the concentration of the nitrogen and phosphorus in the plant tissues. Wondimu and Tana (2017) reported that increasing the rate of N and P in common beans increased their concentration in the tissues. This study determined the effect of integrating NAA, staking, N and P on protein and phosphorus content of the climbing bean seed.

MATERIALS AND METHODS

Study Site

The study was carried out at Kaguru Agricultural Training Centre (Kaguru-ATC), located in Imenti South Sub county of Meru County, Kenya, at latitude, $0^{0}05'$ South and longitude $37^{0}40'$ East. The average temperature range is 16 – 23 ^{0}C and lies at an altitude of 1517 m above sea level. The site has well drained, deep dark reddish-brown friable clay i.e. humic nitosols. The rainfall is bimodal with long rains occurring between March and May and short rains between October and December of every year.

Experimental Design

The experiments were laid out in a Randomised Complete Block Design and replicated three times. Three factors, that is, NAA concentrations at three levels (0, 0.2 and 0.4 mlL^{-1}), staking at three levels (no staking, staking with stakes and maize plants as support) and Diammonium phosphate (DAP) fertiliser at four levels (0, 200, 250 and 300 Kg $DAPha^{-1}$) were tested. The DAP was the source of N (equivalent of 0, 36, 45 and 58 Kg Nha^{-1}) and P (equivalent of 0, 92, 115 and 138 Kg Pha^{-1}). The factors and their levels combination constituted 36 treatments. The experimental field was divided into three blocks and each block was then divided into 36 experimental plots. Each experimental plot measured 2.5 by 2.7 m consisted of 5 rows of climbing beans and each row had 9 plants therefore making a total of 45 plants per each experimental plot.

Field Trials

Climbing beans were planted over three growing seasons; season 1 (March – July 2017), season 2 (October 2017 to February 2018) and season 3 (October 2018 to February 2019). The treatments composed of four levels of NAA (0, 0.2 and 0.4 mlL^{-1}) applied twice during the growing period. The first application was done fourteen days after crop emergence while the second application was done at the onset of flowering and shielding with tarpaulin was done to avoid NAA spray drift. The second treatment was 3 staking methods (using stakes, use of maize as stake, no staking). Stakes measuring 3.5 m were pegged next to the bean plants twenty one days after planting and the maize for supporting bean plants was planted seven days before planting of beans at a spacing of 75 by 25 cm. The DAP fertilizer supplying inorganic nitrogen and phosphorus was applied at planting time.

Data Collection

After harvesting, laboratory analysis was done to determine seed phosphorus, nitrogen and crude protein content. Twenty seeds were picked at harvesting stage from the experimental plants from every plot. These seed samples were grounded and then digested using a ratio of 1:1 concentrated sulphuric acid and Hydrogen peroxide a process carried out in the laboratory. The P-content in the seed tissue samples was determined according to Olsen and Sommers (1982) method. The Nitrogen content was measured by the micro-kjeldah digestion method (Bremner and Mulvaney, 1982). After obtaining the Nitrogen content through the laboratory analysis the results obtained were multiplied by a factor of 6.25 to get crude protein percentage (Bremner and Mulvaney, 1982).

Data Analysis

The data collected were subjected to analysis of variance using Statistical Analysis Software (SAS) version 9.4. The significant means were separated using Least Significant Difference (LSD) at $\alpha = 0.05$.

RESULTS

The results of this study showed that there was a significant effect ($p<0.05$) of treatments on the seed N, P and crude protein content of the climbing bean (Table 1). The analysis of individual factors revealed that there was significant effect ($p < 0.05$) of application of NAA and fertiliser, and staking on P, N and crude protein content of harvested climbing bean seeds (Table 2). The seed percent P content ranged from 0.16 % to 0.62 %, while the seed percent N and crude protein content ranged from 2.07% to 4.89% and 12.96 % to 30.54 %, respectively (Table 3). The application of 0.4 mlL^{-1} NAA and 300 Kg $DAPha^{-1}$ and supported by maize stakes gave the highest percent seed P, N and crude protein content.

The application of 0 mlL^{-1} NAA and $0KgNha^{-1}$ and unsupported gave the lowest percent seed P, N and crude protein content. Analysis was also performed for each of the factors tested (Table 4). The results clearly showed that application of 0.4 mlL^{-1} NAA gave significantly ($p < 0.05$) highest percent seed P, N and crude protein compared to other levels of NAA application. Similarly, staking with stakes performed significantly better than other staking types, except for seed crude protein where there was no significant different between staking with stakes and supporting beans with maize plants. Generally, the amount of seed P, N and crude protein increased with increased rates of fertiliser application (Table 4). There was a strong correlation ($r = 0.924$, $p < 0.001$) between seed nitrogen content and seed phosphorus content.

Table 1: Analysis of variance for effect of NAA, staking, and application of inorganic N and P on seed P, N and crude protein of climbing beans

Variable	Source of Variation	df	SS	MS	F-value	p-value
Seed phosphorus	Block	2	0.0180056	0.0090028	3.01	0.0556
	Treatment	35	1.5013667	0.0428962	14.35	<0.0001
Seed nitrogen	Block	2	5.9386889	2.9693444	32.27	<0.0001
	Treatment	35	76.246825	2.1784807	23.68	<0.0001
Seed crude protein	Block	2	220.39265	110.196343	28.73	<0.0001
	Treatment	35	2910.7318	83.163767	21.68	<0.0001

Table 2: Analysis of variance of the effect of factors on phosphorus (P), nitrogen (N) and crude protein (CP) content of harvested climbing beans

Variable	Source of variation	df	SS	MS	F-value	p-value
P	Block	2	0.01800556	0.00900278	3.01	0.0556
	NAA	2	0.92120556	0.46060278	154.13	<0.0001
	Staking	2	0.16671667	0.08335833	27.89	<0.0001
	DAP	3	0.34047778	0.11349259	37.98	<0.0001
	Auxin*Staking	4	0.01172778	0.00293194	0.98	0.4236
	Auxin*DAP	6	0.04395000	0.00732500	2.45	0.0329
	Staking*DAP	6	0.00635000	0.00105833	0.35	0.9052
	Auxin*Staking*DAP	12	0.01093889	0.00091157	0.31	0.9865
N	Block	2	5.93868889	2.96934444	32.27	<0.0001
	NAA	2	56.91190556	28.45595278	309.29	<0.0001
	Staking	2	12.72937222	6.36468611	69.18	<0.0001
	DAP	3	4.75695833	1.58565278	17.23	<0.0001
	Auxin*Staking	4	0.55390556	0.13847639	1.51	0.2101
	Auxin*DAP	6	0.93178333	0.15529722	1.69	0.1368
	Staking*DAP	6	0.19185000	0.03197500	0.35	0.9090
	Auxin*Staking*DAP	12	0.17105000	0.01425417	0.15	0.9995
CP	Block	2	231.980035	115.990017	32.27	<0.0001
	NAA	2	2223.121311	1111.560655	309.29	<0.0001
	Staking	2	497.241102	248.620551	69.18	<0.0001
	DAP	3	185.818685	61.939562	17.23	<0.0001
	Auxin*Staking	4	21.636936	5.409234	1.51	0.2101
	Auxin*DAP	6	36.397786	6.066298	1.69	0.1368
	Staking*DAP	6	7.494141	1.249023	0.35	0.9090
	Auxin*Staking*DAP	12	6.681641	0.556803	0.15	0.9995

Table 3: Effect of NAA, staking and application of inorganic N and P on seed N, P and crude protein content of climbing beans

Treatment	Seed phosphorus	Seed nitrogen	Seed crude protein (%)
A3S2F4	0.6240a	4.7500a-c	29.64a-c
A3S2F3	0.5738ab	4.6500a-c	29.07a-c
A3S2F2	0.5135b-d	4.4600ab-e	27.8ab-e
A3S3F4	0.5470a-c	4.8867a	30.54a
A3S2F1	0.3570g-m	4.2700c-f	26.69c-f
A3S3F3	0.5067b-d	4.8500ab	30.31ab
A2S2F4	0.4700c-e	4.3700b-f	25.32d-g
A3S1F4	0.4600c-f	3.8867f-i	23.73f-i
A2S2F3	0.4333d-g	4.1767d-g	26.52c-f
A3S3F2	0.4700c-e	4.300c-f	26.87b-f
A3S3F1	0.3667g-l	4.0033e-h	25.02e-h
A2S2F2	0.4167e-h	4.033e-h	25.21e-h
A3S1F3	0.4167e-h	3.8867f-i	24.29f-i
A2S3F4	0.4367d-g	4.3700b-f	27.31ab-e
A2S2F1	0.2833k-p	3.7200g-j	23.25g-j
A1S2F4	0.2833k-p	2.8833k-m	18.02k-m
A3S1F2	0.400e-i	3.5700h-j	22.32h-j
A2S3F3	0.4057e-i	4.2833c-f	26.78c-f
A1S2F3	0.2967j-p	2.7200mn	17.00mn
A2S3F2	0.3800f-j	3.9700ef-h	24.81e-h
A1S3F4	0.30000j-o	2.8367l-n	17.73lmn
A2S1F4	0.3767f-k	3.5600h-j	22.24h-j
A1S2F2	0.2700l-q	2.6733m-o	16.71m-o
A2S3F1	0.2900k-p	3.5367h-j	22.73h-j
A2S1F3	0.3400h-m	3.4567ij	21.61ij
A1S2F1	0.2300n-q	2.6700m-o	16.69mo
A1S3F3	0.2433m-q	2.6800m-o	16.75mo
A2S1F2	0.3233i-n	2.6667j-l	20.41j-l
A3S1F1	0.2833k-p	3.3700jk	21.06jk
A2S1F1	0.2233n-q	2.7200mn	17.00mn
A1S1F4	0.2367m-q	2.3433n-q	14.65n-q
A1S1F3	0.2100opq	2.2067o-q	13.80o-q
A1S3F2	0.2133no-q	2.6100m-p	16.31m-p
A1S3F1	0.1833pq	2.5267m-q	15.71m-q
A1S1F2	0.1867pq	2.1167o-q	13.23pq
A1S1F1	0.1567q	2.1167pq	12.96q
LSD	0.089	0.49	3.19
CV%	15.496	8.61	8.92
R^2	0.88	0.93	0.93

[a]Means followed by the same letters are not significantly different at 5% probability level. Where, A=NAA (A_1; 0 $mlL^{-1}$$A_2$; 0.2 mlL^{-1}, A_3; 0.4 mlL^{-1}); S= Staking (S_1; No staking, S_2; Staking using stakes, S_3; maize for support); F=DAP [F1; 0 Kg $DAPha^{-1}$ (0 Kg Nha^{-1}; 0 Kg Pha^{-1}); F_2; 200 Kg $DAPha^{-1}$ (36 Kg $Nha^{-1;}$ 92 Kg Pha^{-1}); F_3; 250 Kg $DAPha^{-1}$ (45 Kg $Nha^{-1;}$ 115 Kg Pha^{-1}); F_4; 300 Kg $DAPha^{-1}$ (58Kg$Nha^{-1;}$ 138KgPha^{-1})].

Table 4. Percentage mean of phosphorus (P), nitrogen (N) and crude protein (CP) content under different factors (auxin, types of staking and fertiliser)

Factor level	P	N	CP

Auxin levels			
0.4 mlL^{-1} NAA	0.4594a	4.2403a	26.5017a
0.2 mlL^{-1}NAA	0.3647b	3.8006b	23.7535b
0 mlL^{-1} NAA	0.2342c	2.5283c	15.8021c
LSD	0.0257	0.1426	0.8912
Staking types			
Staking using stakes	0.3958a	3.7850a	23.6563a
maize for support	0.3617b	3.7461a	23.4132a
No staking	0.3008c	3.0381b	18.9878b
LSD	0.0257	0.1426	0.8912
Fertiliser levels			
300 Kg $DAPha^{-1}$	0.4148a	3.7700a	23.5625a
250 Kg $DAPha^{-1}$	0.3804b	3.6567a	22.8542a
200 Kg $DAPha^{-1}$	0.3526b	3.4444b	21.5278c
0 Kg $DAPha^{-1}$	0.2633c	3.2211c	20.1319c
LSD	0.0297	0.1646	1.0291
CV (%)	15.50	8.61	8.61
R^2	0.88	0.93	0.93

[a]Means followed by the same letters are not significantly different at 5% probability level. Where, 200 Kg $DAPha^{-1}$ provides 36$KgNha^{-1;}$ 92 $KgPha^{-1}$); 250 Kg $DAPha^{-1}$ provide 45$KgNha^{-1;}$ 115$KgPha^{-1}$; and 300 Kg $DAPha^{-1}$ provides 58$KgNha^{-1;}$ 138$KgPha^{-1}$.

DISCUSSION

The findings of the study revealed that the amount of NAA applied influenced the amount of seed N, P and crude protein content of the harvested bean seed. The treatments with 0.4 mlL^{-1} NAA concentration recorded the highest percent seed P, N and crude protein content of the climbing bean plant, implying that the optimum amount of NAA to get the maximum improvement of these attributes have not been achieved.

Bairwa and Mishra, (2017) reported that NAA could increase the osmotic uptake of water and nutrients. Probably the NAA stimulated increase in permeability of membrane to uptake of water and nutrients including inorganic N and P, leading to increased accumulation of the N and P in the plant tissues including the seeds. Seed nitrogen concentration is directly proportional to seed crude protein, therefore any factor that increases seed nitrogen content will improve the seed protein quality. Nascimento and Mosquim, (2004) reported that auxins stimulates protein synthesis in soya bean. Application of inorganic N and P was found to significantly affect the seed N and P content of the climbing bean.

Belinger et al., (2012) reported that increases in N content due to N fertilization led to increase in seed P content. Moreover other studies have shown a positive correlation in increase of P and N rate of application to increases in their concentrations in the plant tissues of the common beans (Wondimu and Tana, 2017). This could be attributed to increase concentration of nitrogen and phosphorus elements in the soil solution, which in turn improved plant growth and development thereby increasing nutrient uptake (Fageria, 2009). Staking had a significant influence on nutrient N and P uptake. Staking stimulates high vegetative growth that probably creates a demand for nutrient N and P, therefore, leading to more uptakes of these nutrients that eventually accumulated in seed and plant tissues.

Sperling and Muyaneza, (1995) reported that climbing beans require more nutrient input because of their larger biomass production. However, the nitrogen and protein content of the bean seed was higher for the bean plants supported by the maize plants. Probably high accumulation of mineral N in the climbing bean plants supported by maize plants was due to less vigorous growth and probably led to less utilization of nutrient N and therefore more accumulation in the seed tissue of the climbing bean. Agronomically, seed quality can be improved by managing seed crops to aid accumulation of mineral nutrients through application of NAA, staking and mineral N and P. Seed quality is important for nutritional value and stand establishment in production of climbing beans.

CONCLUSION

The results of this study indicated that application of NAA, N and P and staking affected the seed P, N and crude protein content of the climbing beans. The differences in these seed quality were obtained despite using the same seed lot and similar seeding rates among the different treatments. Thus, differences in these seed quality attributes were the outcome of variation in treatments. An increase in amount of NAA resulted in a significant increase in amount of seed N, P and crude protein content. This shows that the optimum amount of NAA to be applied to optimize these seed content was not arrived at this experiment. The study also demonstrated the importance of staking climbing beans as this led to improved seed quality of the climbing bean. Therefore integration of NAA, staking, P and N application lead to enhanced uptake of soil nutrients which resulted to improved seed quality of climbing beans.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Abd, B., Abd, R., MON, E., & Kassem, A, (2018). Response of Growth, yield and Nutritional Status of Cucumber plants (*Cucumissativum*) to different foliar application of humic acid and naphthalene acetic acid.*Middle East Journal of applied sciences, 6 (2): 594 – 598.*

Bairwa, S. & Mishra, J. S. (2017). Effect of NAA, BA and Kinetin on yield of African marigold (Tageteserectalinr). *Introduction Journal Microbiology Application Science,* 6; 1236-1241.

Belanger, G., Cllaessens, N. & Ziadi, N. (2012). Grain N and P relationships in maize. Field Crops Research: 126: 1-7.

Bremner, J. M. & Mulvaney, C.S. (1982). Methods of soil analysis, part 2 chemical and microbiological properties, 595 – 624.

Fageria N. K. (2009) The use of nutrients in crop plants. CRC press, *Taylor & Francis group, New York, USA*, pp. 1-127.

Faizan, M., Faraz, A., Sami, F., Siddiqui, H., Yusuf, M., Gruszka, D., and Hayat, S. (2020). Role of strigolactones: Signalling and crosstalk with other phytohormones. Open Life Sci. 2020; 15: 217–228

Finch – Savage, W. & Bassel B. W. (2015). Seed vigour and crop establishment: extended performance beyond adaptation. *Journal of experimental Botany*, vol. 67, issue 3, 567 – 591.

Hasan, M., Robbani, M., Parvin, R., Mehedi, M.N.H., and Hossain, I. (2019). Effect of NPK on Growth, Yield and Seed Quality of Hybrid Chilli. Int. J. Agril. Res. Innov. Tech. 9 (1): 35-41.

Karim, F., Fattah, Q. & Khaletozzaman, M. (2006). Changes in biocomponents of chickpea (Cicerarentinum 1). Sprayed with potassium Naphthanete and Naphthalene acetic acid. *Baghladesh Journal Botanical*, 35 (1): 39-43.

Lwakuba, A., Kaudia, A. A., Okorio, J, Esegu, J. F., &Oluka, A. I. (2003). *Agroforestry handbook for the montane zone of Uganda. RELMA Technical Handbook No. 31*. Regional Land Management Unit (RELMA), Swedish International Development Cooperation Agency (Sida). Nairobi, Kenya. pp 33-34.

Martínez-Ballesta, M. del Carmen., Egea-Gilabert, C., Conesa, E., Ochoa, J., Vicente, M.J., Franco, J.A., Bañon, S., Martínez, J.J., & Fernández, J.A. (2020). The Importance of Ion Homeostasis and Nutrient Status in Seed Development and Germination. Agronomy, 10, 504; doi:10.3390/agronomy10040504

Moussavi, N. (1997). Seed quality and crop establishment in wheat. Ph.D Thesis. University of Aldelaide, department of plant science.

Nascimento, R. & Mosquim, P. R. (2004). *Cresscmento e teor de prokinasemsementes de soja sob influencia de hormoniosvegetais. RevistaBrasileira de Botanica*, v. 27, p. 573 – 579.

Ojo E, Ajiboye AA., Fawibe OAB, Ajewole TO., Akinloye O and Agboola D (2017) Effects of Phytohormone on Seed Germination, Seedling Vigour and the Phytochemical Contents of Three Cucurbits. Asian Journal of Crop Science, 9: 63-70.

Olsen, S.R. and Sommers, L.E. (1982) Phosphorus. In: Page, A.L., Ed., Methods of Soil Analysis Part 2 Chemical and Microbiological Properties, American Society of Agronomy, Soil Science Society of America, Madison, 403-430.

Rajala A, Niskanen M, Isolahti M and Peltonen-Sainio P (2011) Seed quality effects on seedling emergence, plant stand establishment and grain yield in two-row barley. Agricultural and Food Science, Vol. 20: 228–234.

Sperling, K. & Muyaneza, S. (1995). Intensifying production among smallholders: The impact of improved climbing bean in Rwanda. *African crop science Journal* 3: 117 – 125.

Wondimu, W. & Tana, T. 2017. Common beans (Phaseolus vulgaris L.) varieties to combined application of N and P fertilisers at Mechar, Eastern Ethiopia J Plant Biol Soil Health: 4 (2): 7