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Original Article

Polyploid induction in selected Mung Bean (*Vigna radiata* L. Wilczek) genotypes

OPEN ACCESS

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ABSTRACT

This experiment was conducted to evaluate the effects of different immersion periods of colchicine 0.2% solution on the seeds of three mung bean accessions. The experiment was a 3×3 factorial experiment in complete randomized design and the significant means were separated using least significant difference (LSD) at 5% probability level. The experiment was replicated three times. The immersion periods were: 2 and 4 hours while ordinary water was used as control and the accessions of mung bean were Tvr 72, Tvr 77 and Tvr 98. The different mung bean accessions and their seeds immersion periods in colchicine 0.2% solution showed no significance difference ($p \leq 0.05$) on days to germination, emergence and 50 % flowering respectively. The three mung bean accessions in two hours immersion relatively produced higher values of all the measured parameters than 4 hours immersion. The number of leaves, plant heights, stem girth and number of pods did not significantly vary in Tvr 77 and Tvr 98 cochiploids of two hours immersion. The Tvr 77 although closely followed by Tvr 98 produced the largest measured leaf area (192cm2) and longest pods (11cm) and largest number of seeds (15). We therefore recommend the multiplication and use of Tvr 77 and Tvr 98 cochiploids for mung bean seed production and further breeding.

K E Y W O R D S : *Accessions, Breeding, Cochiploids, Colchicine, Mung bean, Seeds*

INTRODUCTION

Mungbean (*Vigna radiata* L. Wilczek) is a pulse that belongs to the Legumes family *Fabaceae*. Legumes are grown primarily for human consumption, livestock forage and silage, and as soil-enhancing green manure (Elbert, 2014,Das *et al*.,2014). It is a short duration legume, which can be grown as a sole or with other crop under rain fed and irrigated conditions. It is an excellent source of easily digestible high quality protein for the predominant vegetarian population.(Obasi, *et al*.,2024,Tomooka *et al*.,2003)It contains 3.5-4.5% fiber, 22-28% total protein, 21-25% of total amino acid and 1.53-2.63% lipids, 1.0-1.5% fat, ash content ranges from 4-5% and 59-65% carbohydrate on dry weight basis and provide 334-344 kcal energy (Idoko & Avav, 2003, Aguogu, 2010). It is

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rich in various B vitamins and also high in protein and dietary fibre (DOA., 2013).it also serves for medicinal purpose (Hujjie *et al*., 2003, Olunike, 2014). Due to good taste easy digestibility, better palatability and acceptable market price may be the first choice of farmers (Aguogu, 2017). It increases farmers income and improves soil fertility through symbiotic nitrogen fixation (Malik *et al.*, 2000).Other than food it is important for assistance in normal use of land water resources and enriching the soil. Polyploidization of plants occurs in nature but it is a slow and gradual process that has driven evolution and speciation throughout the ages (Sattler *et a*l, 2015). As time is important, polyploidization can be induced in a shorter period by using colchicine that interferes with mitosis of cells(Eng & Wei,2019).Colchicine is a toxic chemical that is often used to induce polyploidy in plants. Basically, the colchicine prevents the microtubule formation during cell division, thus the chromosomes do not pull apart like they normally do. The end result is a cell that now has double the number of chromosomes that it would normally have (Sattler *et a*l,2015). If this cell divides again in the future, then the doubled number of chromosomes are passed to the offspring cells(Godfree *et al*.2017). Plants that have more than the normal two sets of chromosomes are termed "polyploids" in general, although specific names are given to the certain chromosome numbers (e.g. tetraploid or 4N plants have four sets of chromosomes) (Eng & Wei, 2019). Polyploid plants are generated in an effort to create new plants that have new characteristics (Saxena *et a*l.2014). Sometimes the polyploidy plants are sickly and not viable, but sometimes the polyploid plants have larger leaves,number of leaves, flowers and fruits (Sattler *et al*,2015. The aim of the project is to induce polyploidy in mungbean seeds using a given concentration of colchicine with different immersion periods.The produced cochiploids are expected to have larger number of leaves,bigger leaves,flowers,pods and seeds, there by increasing productivity and survival(Godfree *et al.*,2017).

MATERIALS AND METHODS

Experimental site

The work was done at University Teaching and Research Farm.Nnamdi Azikiwe University, Ifite-Ogwari Campus, Anambra State. Ifite-Ogwari is located within latitude 6^0 16'N and longitude 7^0 7'E with an altitude of 422m and an average rainfall of 1650mm to 1824 per annum, The minimum and maximum temperatures in the area are 27^0C and 32^0C respectively and a relative humidity of 75-80%. The rainfall distribution is bimodal; between April and July and between September and November with a short break in August (NIMET, 2022).

Source of Materials

The three genotypes of Mung bean viz :Tvr 72, Tvr 77 and Tvr 98 that were used for this research were collected from the International Institute of Tropical Agriculture (IITA), Ibadan.Colchicine was sourced from Quality chemicals Ltd Onitsha.

Experimental materials and preparation

The Experiment involves both laboratory and field works. One gram of Colchicine was dissolved in 499mls of distilled water to give 500mls or 0.2%Colchicine solution,The colchicine solution was then divided into two batches of 250mls each.About 50 mung bean seeds were immersed in one of the 250mls container for 2 hours as batch A while Batch B was immersed for 4 hours .The control seeds were immersed in distilled water .The seeds were then be removed and placed in between moist filter paper and kept for germination. After about 4 days, the germinated seeds were planted in nursery bags with normal nursery soil mixture.They were randomly arranged at 50 cm inter and intra rows spacings.

Agronomic practices and Data collection

Normal agronomic activities were observed and data collected include:, leaflet length and width, plant height Number of leaves per plant, number of branches per plant, number of stems per plant, Days to first emergence, days to first flowering, days to 50% flowering, number of flowers per plant, Number of Pods Per Plant, Pod length(cm),Pod weight(g), and Number of seeds per pod.

Treatments

Factor A: The treatments consisted of A = control, B = 2 hours, C = 4 hours immersions.

Factor B: Different varieties of mung bean. (TVR72, TVR77, TVR98)

Experimental design

The experimental design was a 3 x 3 factorial experiment in a complete randomization design (CRD) with three replications. Factor A were three (3) colchicine immersion periods(2hrs,4hrs and control) whereas Factor B were three (3) genotypes of mung bean (TVR72, TVR77, TVR98).

Statistical analysis

The data obtained from the various observations, were subjected to statistical analysis by using analysis of variance while differences of the treatment of means were separated using least significant difference at 5% level of probability. General analysis of variance (ANOVA) for the recorded data was performed using GenStat 10.3 Discovery Edition (Genstat, 2012) software to establish

differences among the accessions with regard to the quantitative estimates of the morphological traits.

RESULTS AND DISCUSSION

The effect of colchicine treatments on cochiploids germination, emergence and days to 50% flowering.

Table 1. showed that the different mung bean accessions and their seeds immersion periods in colchicine 0.2% solution had no significance difference ($p \leq 0.05$) on days to germination, emergence and 50 % flowering respectively as showed on table 1. Although, the 4 hrs immersion relatively delayed germination to 3 days, emergence to 7 days and days to flowering to 39 days. This was in accordance with the findings of Harbard,*et al.*, (2012). A non- significant effect of colchicine on germination and survival percentage of black wattle (*Acacia mearnsii*) was observed when its seeds were grown on colchicine saturated filter papers.

Table 1:The effect of colchicine treatments on cochiploids germination, emergence and days to 50% flowering.

Treatment combination	Days to emergence	Days to germinate	Days to 50% flowering
TVR72	4.56	2	31.44
TVR77	5.11	2	32.22
TVR99	4.69	2	31.67
LSD (0.05)	NS	NS	NS
Control	2.22	1	30.44
2hours	5.56	2	26.33
4hours	6.98	3	38.56
LSD (0.05)	NS	NS	NS

Effect of colchicine treatments interactions on days to emergence, days to germination and days to 50% flowering.

The treatment interactions as seen on Table 2 showed no significance ($p \leq 0.05$) on days to emergence, days to germinate and days to 50% flowering. Although, the 4 hrs immersion despite mung bean cultivars relatively delayed germination to 3 days, emergence to 7 days and days to flowering to 39 days. This was also on line with the findings of Harbard et al., (2012) who discovered a non-significant effect of colchicine on germination and survival percentage of black wattle (*Acacia mearnsii*) was observed when its seeds were grown on colchicine saturated filter papers. .

Table 2: Effect of colchicine treatments interactions on days to emergence, days to germination and days to 50% flowering.

Treatment	Days to emergence	Days to germination	Days to 50% flowering
TVR72 + control	2.00	1	31.00
TVR 77 + control	2.67	1	31.33
TVR98 + control	2.00	1	29.00
TVR72 + 2hr	5.33	2	26.33
TVR77 + 2hr	5.67	2	26.33
TVR98 + 2hr	5.67	2	26.33
TVR72 + 4hr	6.33	3	37.00
TVR77 + 4hr	7.00	3	39.00
TVR98 + 4hr	7.00	3	37.67
LSD (0.05)	NS	NS	NS

Cochiploids growth parameters.

There was no significance difference amongst the mung bean accessions heights. The 2 hrs immersed seeds produced tallest plants (29cm) followed by control (27cm) on Table 3.The least height came from 4hr immersed(16cm).Tvr77 and Tvr 98 produced the largest number of branches (5) followed by Tvr72.Table 3 showed that there was no difference in the accessions number of leaves while 4 hr immersion produced the least number of leaves (18).Tvr 77 produced the largest measured leaf area (134.6cm^2) followed by Tvr 72 (111.1cm^2). .Tvr 98 was the least (102.6cm^2).There was no significance difference on stem girth, although,Tvr 77 produced the biggest girth(1.44cm^2)..

Table 3: Cochiploids growth parameters

Treatment combination	Height (cm)	Number of branches	Number of leaves	Size of leaves	Stems
TVR72	23.98	4.89	26.22	111.10	1.42
TVR77	24.78	5.11	26.67	134.60	1.44
TVR98	23.89	5.33	25.78	102.60	1.33
LSD (0.05)	1.235	0.603	1.009	6.97	0.244
Control	27.44	6.00	29.56	141.10	1.00
2hrs	29.11	6.22	30.44	161.60	2.00
4hrs	16.00	3.11	18.67	45.60	1.20
LSD	1.235	0.003	1.009	6.960	0.244

Colchicine treatments interactions on cochiploids growth parameters.

On growth parameters treatment interactions, 2hrs immersion produced the highest plants(29cm) followed by control (27.44cm). Tvr 77 under control,produced the tallest plants(29cm) .The least came from 4hrs immersion.The three accessions had the same number of branches (6) in the control. The 4hrs immersion produced the least number of branches (3).Also the 2hrs immersion produced plants with the largest number of leaves across the accessions followed by control. The least still came from the 4hrs immersion. The largest leaf size(192cm^2) came from Tvr 77 in 2hrs immersion followed by the same Tvr 77 control (164cm^2) and Tvr 72 (154cm^2) which did not significantly vary.The 2-hour colchicine treatment generally enhanced growth parameters, such as height, number of branches and leaves.This is due to doubling of chromosome number.This is in accordance with the findings of Sattler *et al.*,(2015). The least came from 4hrs immersion. The widest stem girth came from the 2hrs immersion and were the same in the accessions (2cm).This is due to increase in ploidy number. This is in line with the findings of Tiwari & Mishra, (2012) in colchicine treated phlox (*Phlox drummondii*) plants, increase in size of polyploidy cells maximizes the stomatal dimensions. Cells with larger genomic material grow bigger to retain the constant ratio between the cytoplasmic and nuclear volume and enhance the expression of proteins due to the increase in number of genes. This increase in cell size may result in the development of larger plants (Tiwari & Mishra, 2012).Their number of leaves,plant heights,stem girth and number of pods did not significantly vary. The 4hrs immersed seeds had stunted growth and reduced leaves sizes,this might be due to long exposure time to colchicine solution. As related by Ari *et al.*, (2015) Colchicine significantly decreases the number of leaves per plant, leaf area, length and width but it increases leaf thickness in 0.3% concentrations. This may be due to reduction in cell division that retard the growth or it may occur due to hormonal imbalances.Tvr 77 although closely followed by Tvr 98 produced the largest measured leaf area (192cm^2) and longest pods (11cm) and largest number of seeds(15).These increase might be due to increase in ploidy level.In tetraploid plants of arabidopsis (*Arabidopsis thaliana*), enhanced growth was associated with increase in ploidy level. This increase doubled the DNA content for gene expression which in turn improves the metabolic activity of cells thus promotes the growth rate (Sattler *et al,2*015).The control and 4hrs immersion had the same girth (1cm).

Table 4:Colchicine treatments interactions on cochiploids growth parameters.

Treatment combination	Height (cm)	Number of branches	Number of leaves	Size of leaves	Stem girth
TVR72+control	26.67	5.67	30.00	133.3	1.00
TVR77+control	29.33	6.00	29.00	164.7	1.00
TVR98+control	26.33	6.33	29.67	125.3	1.00
TVR72 + 2hr	29.33	6.00	29.33	154.0	2.00
TVR77 + 2hr	29.10	6.00	31.67	192.0	2.10
TVR98 + 2hr	29.00	6.67	30.33	138.2	2.00
TVR72 + 4hr	15.67	3.00	19.33	46.00	1.267
TVR77 + 4hr	16.00	3.33	19.33	46.70	1.33
TVR98 + 4hr	16.33	3.0	19.33	44.00	1.00
LSD (0.05)	2.139	1.04	1,747	12.06	0.42

Cochiploids yield parameters

Table 5 showed that both Tvr 77 and Tvr 98 had the same number of flowers,number of pods and seeds per pod but Tvr 77 had the longest pod(8.22cm). The 2 hrs immersed accessions produced the highest number of flowers (10.44), number of pods (18), number of seeds per pod(14) and pod lenght(9.8) these were followed by the control treatment while the 4hrs immersion produced the least of the measured parameters. Sattler *et al.,(*2015) also noticed increased yield parameters like pod size,seed size and number etc on cochiploids.

Table 5: Cochiploids yield parameters

Treatment combination	Number of flower	Number of pods	Number of seeds per pods	Pod length
TVR72	77.11	13.22	10.44	7.10
TVR77	8.33	14.00	11.22	8.22
TVR99	8.22	13.22	10.44	7.00
LSD (0.05)	**0.738**	**1.826**	**0.852**	**0.938**
Control	9.11	18.89	11.67	8.67
2hours	10,44	18.89	14.00	9.88
4hours	4.11	5.67	6.44	4.00
LSD (0.05)	**0.735**	**1.026**	**0.852**	**0.738**

Cochiploids yield parameters interactions.

On interactions, Table 6 showed the effects of the treatment combinations, Tvr 98 immersed for 2hrs produced the largest number of flowers(11) followed by Tvr77(10.67).The controls were not significantly different while the least values came from 4hrs immersion. On number of pods, the accessions at 2hrs immersion produced the same number of pods which were more than that in control while the 4hrs immersed produced the least. Tvr77 and 98 produced the largest number of seeds followed by Tvr72 at 2hrs immersion.This is due to increase in ploidy number.The controls also produced more number of seeds per pod than the 4hr immersion. Tvr77 at 2hrs immersion produced the longest pod(11cm) followed by the same Tvr77 in control(9.67cm).The least values still came from the 4hrs immersion.

Table 6: Cochiploids yield parameters interactions.

Treatment combination	Number of flower	Number of pods	Number of seeds per pods	Pod length
TVR72 + control	8.33	16.00	11.67	8.67
TVR 77 + control	9.33	15.00	11.67	9.67
TVR98 + control	9.67	16.00	11.67	7.67
TVR72 + 2hr	9.67	19.00	13.00	8.67
TVR77 + 2hr	10.67	19.33	15.00	11.00
TVR98 + 2hr	11.00	18.33	14.00	9.10
TVR72 + 4hr	5.33	4.67	6.67	3.67
TVR77 + 4hr	5.00	7.00	7.00	4.00
TVR98 + 4hr	4.00	5.33	5.67	4.32
LSD (0.05)	1.279	1.778	1.426	1.279

CONCLUSION AND RECOMMENDATION

Colchicine immersion periods had no effect on mung bean germination and emergence.The two hours colchicine immersion produced the highest of the measured mung bean parameters.While the four hours colchicine immersion produced cochipliods with stunted growth and thick leaves.The two hours immersion produced cochipliods of Tvr 77 which were closely followed by Tvr 98 had the largest measured leaf area,longest pod and largest number of seeds.We therefore recommend the multiplication and use of Tvr 77 and Tvr 98 cochiploids for mung bean seed production and further breeding.

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Authors's contribution

Authors OAU & HO managed data collection, interpretation of data and writing of manuscript, CCO & SNO sourced the cultivars and review of manuscripts. GCA did type setting, EAO managed the literature searches and data analysis, development of methodology and data analysis. All authors read and approved the final manuscript.

Ethical Approval

Not Applicable

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