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MUTATION BREEDING IN COWPEA VIGNA UNGUICULATA (L.) WALP. (FABACEAE)

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ABSTRACT:

The mutagenesis study was carried out on the seeds of cowpea (*Vigna unguiculata* L.Walp.) of variety PARVATI-AV 89 which was collected from the local market to induce mutation. The seeds were exposed to varying doses of chemical mutagens for different duration of time namely Ethyl Methane Sulphonate (EMS) and Sodium Azide (SA) of concentration (0.01,0.02,& 0.03%) and (0.05,0.10,0.15%) respectively. The M1 generation of treated seeds and mutants were observed or screened phenotypically based on their morphological characters and compared with the controlled plants. An increased production in the average length of pods and survival of plants at maturity was observed and it was found more in the plants, treated with mutagenic agent with the concentration of 0.01% and 0.02% EMS for 4hrs as compared to other doses of SA and control according to the set objectives.

Keywords: Mutagenesis, EMS, SA, Mutants, cowpea, *Vigna unguiculata* (L.)Walp. Var.PARVATI-AV- 89.

INTRODUCTION:

Cowpea *Vigna unguiculata* (L.) Walp. is one of the most important pulse crop in tropical Africa and other countries as well where it constitutes a valuable source of protein in the diets of millions of people.It belongs to the family Fabaceae ,tribe phaseoleae genus *Vigna* and section catiang(Marechal,Mascherpa and Stainer ,1978)(1) . Mostly the seeds are the major source of dietary protein in most of the developing countries (Duke 1990) (2). Cultivated cowpea, which is in subspecies *unguiculata*, divided into five cultivar groups namely *unguiculata,sesquipedalis (Yard- long- bean),textilis, biflora and melanophthalmus.* Commonly cultivated cowpea belongs to cultivar group *unguiculata* while other members are grown in some part of Nigeria for production fibre (3). In Nigeria, crop breeding through induced mutation is limited (Odeigah1991)(4).Here we are focusing on mutation breeding for crop improvement. Cowpea is diploid with 2n =22 and a genome size of about 620 million base pairs. Cowpea is an annual herb with varying growth forms. It may be erect trailing, climbing or bushy, usually indeterminate under favourable conditions. It is primarily used in the form of dry seeds, fodder, green pod, green manure and cover crops. When mutations are induced for crop improvement, the entire operations of the induction and isolation etc of mutants is termed as mutation breeding.(B.D.Singh,2000)(5). Induced mutation breeding which has been recognized as a valuable supplement to conventional breeding in crop improvement has been least applied in grain legumes. For example, only eight out of over 1000 improved mutant varieties of different crops released up to 1989 in over 48 countries were cowpeas (Micke et.al 1990) (5). The present investigation was undertaken to induce

mutation by using chemical mutagen to get the desirable characters which could be utilize directly or introduced into our cowpea improvement programme. Here, we report some of the results or observations on the use of chemical mutagens (EMS and SA) for improvement in the cowpea crop.

MATERIALS AND METHODS:

MATERIALS:

1) Mature and dry seeds of cowpea (*Vigna unguiculata* (L.)Walp. of var. PARVATI – AV -89.

(a) Mutagens :We used two chemical mutagens namely i.e. Ethyl Methane Sulphonate (EMS): It is an alkylating agent .

It is a mutagenic and possibly carcinogenic organic compound with formula $C_3H_8O_3S$. It produces random mutations in the genetic material by nucleotide substitution particularly by Guanine alkylation. Here we used 0.01, 0.02, and 0.03 percent of concentrated solutions of EMS for treating the seeds to induce mutation. (b)Sodium Azide (SA): It induces the chromosomal aberrations. It's molecular formula is NaN_3. SA is known to be highly mutagenic agent in several organisms. Treatment of SA reduces or delays seed germination percent, root length, shoot length and mitotic index. Here we used 0.05, 0.10 and 0.15 percent concentrated solutions of SA for treating the seeds to induce mutation. The utilization of new mutagenic agent in several plant species has Played an important role in mutation breeding (Silva and Barbosa,1996).Among the chemical mutagens, EMS is reported to be the most effective and powerful mutagen(Minocha,1962 and Hajra, 1979)

METHODS:

The mutagen treatment schedule has been given in the table:1, which is explained here. Prior to mutagenic treatment seeds were immersed in distilled water for 6 hrs. The pre-soaking enhances the rate of uptake of mutagen through increase in cell permeability and also initiates metabolism in the seeds. Such pre-soaked seeds were later immersed in the mutagenic solution of EMS and SA for 4 hrs and 5 hrs respectively. Seeds soaked in distilled water for 6 hrs serves as control. Immediately after completion of treatment, the seeds were washed thoroughly under tap water (Osman,*et al*, 2006). Later on seeds with chemical mutagenic treatment were kept for post-soaking in distilled water for 1 hr. For each treatment a batch of 150 seeds of were made. 50 seeds from each treatment were dried in folds of filter paper and germinated in petri dishes to record germination percentage. The remaining 100 seeds from each treatment were sown in field following Randomize Block Design (RBD) with 3 replications along with control as M1 generation.

Table:1 Mutagen Treatment Schedule

Mutagen	**Concentration (%)**	**Presoaking duration (hrs)**	**Treatment Duration (hrs)**	**Postsoaking Duration (hrs)**
EMS	0.01	6	4	1
	0.02	6	4	1
	0.03	6	4	1
SA	0.05	6	5	1
	0.10	6	5	1
	0.15	6	5	1

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Table:2 : Effect of Mutagenic agent on morphology of *Vigna unguiculata:*

Sr. no	Treat-ment	Conc. (%)	No. of seeds sowned	Seed germi-nation (%)	Lethali-ty (%)	Avg seedling height (cm)	Leaf morph ol-ogical Changes (%)	Chlorophyll deficient sector (%)	Pollen viability (%)	Survival of plant at maturity (%)	Average pod length (cm)
1.	Control	Nil	50	100	0	12.66	Nil	Nil	74	80	27.08
2.	EMS	0.01	100	100	0	9.87	30	40	68	66	31.25
		0.02	100	100	0	11.89	50	20	87	94	35.05
		0.03	100	100	0	8.84	50	30	70	88	26.55
3.	SA	0.05	100	50	50	8.95	80	50	86	80	23.58
		0.10	100	100	0	10.16	80	40	73	73	25.58
		0.15	100	100	0	11.89	20	30	57	66	22.38

EXPERIMENTAL RESULTS:

The M1 generation of cowpea was a screened for different biological parameters. The results were recorded & analysed by standard statistical method. The seed germination percent was found to be 100% except 0.05% SA which was 50% with 50 % of lethality. The average seedling height was found to be smaller as compared to control. The leaf morphological changes like tetrafoliate leaves, distorted margin,bifurcation of leaves, change in leaf shape were observed in all treated plants, but the highest ones were observed in 0.05% and 0.10% of SA which was about 80% and the lowest in 0.15% of SA which was 20%. The chlorophyll chimeras were also observed& the highest % of chlorophyll chimeras were observed in 0.05% of SA treated plants& is about 90%, the lowest one is about 30% in plants treated with 0.02% of EMS. The chlorophyll chimeras like Xantha,Viridis and chlorina were observed. Likewise the pollen viability test was also done and the most viable pollens were observed in 0.03% of EMS (87%) as compared with control i.e 74%. The % of survival of plants at maturity increases with increase in concentration in case of 0.02 and 0.03% of EMS which is 94% and 88% respectively. The increased average pod length was also observed in the plants treated with EMS as compared to control and SA. Other than all above mentioned observations the M1 mutants also shows the desirable character like early maturity which was observed in the EMS treated plants of doses 0.01% , 0.02% of EMS as compared to control and SA treated plants. Use of induced mutations for obtaining early maturing cultivars has been a frequent breeding objective (Micke,1979)(6).Likewise some of mutants identified which were showing the difference in their growth habit as compared to control. The observed control plants were showing the medium sized branches whereas some the mutants were showing long branches which were tendril like and were found in both EMS and SA treated plants of concentration of 0.01%, 0.025 and 0.05%,0.10% respectively.

DISCUSSION:

The present investigation shows the mutagenic effectiveness on the different parameters like seed germination percentage, lethality % , average seedling height, leaf morphological changes, chlorophyll deficient sectors, pollen viability ,average length of pods and survival of the plant at maturity etc. The EMS and SA treatment to the seeds shows high germination percentage that is 100% in all the doses except 0.05% of SA which shows 50%

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seed germination.SA is drastically decreasing the seed germination% as earlier mentioned by different workers on mutation breeding. Similarly the used 0.02% EMS concentrated solution shows the most desirable characters which was shown on the observation and result table :2.

CONCLUSION:

Both the mutagens SA and EMS were affected critically on all the parameters in M1 generation of cowpea variety PARVATI-AV-89. The order of mutagenic effectiveness on different biological parameters was studied. The most effective mutagen was found to be 0.02% of EMS in terms of yield and other parameters mentioned in observation table:2. The SA treated plants shows reduction in average pod length as well as decrease in survival of plants at maturity as compared to control plants.

PLATE: MUTAGENIC STUDIES IN COW PEA

Platea. a Chlorophyll mutants

b. change in leaf shape

c. Abnormality in leaf shape

d. tetrafoliate leaf

e. notched margins

f. stunted growth

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