MORPHOLOGICAL STUDIES OF THE EFFECT OF COLCHICINE AND PARADICHLOROBENZENE ON TOMATO (Lycopersicon esculentum)

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ABSTRACT

The seeds of tomato (Lycopersicon esculentum L.) variety Roma VF obtained from Institute of Agricultural Research (IAR), Ahmadu Bello University Zaria, were treated with Colchicine and Paradichlorobenzene with the aim of determining the effectiveness of the mutagens in the production of variations in Tomato. Highly significant difference were observed for germination percentage, number of leaves, and height at maturity. Seedling height, number of branches and germination percentage decrease with increase in concentration of Colchicine and there was increase in leaf length, height at maturity with increase in concentration. While there was highly significant difference in germination percentage, number of leaves, and significant difference in leaf length and height at maturity for Paradichlorobenzene. There was decrease in all the traits that were studied; however, there was increase in seedling height, leaf length, and number of branches with increase in concentration. Paradichlorobenzene showed the highest effect in seedling height, germination percentage at both 5 and 8 days respectively. While colchicines showed the highest effect only in number of branches.Therefore, the use of 1mm and 4mm Colchicine and Paradichlorobenzene is recommended for inducing mutation to create variability and thus increase the possibility of isolating beneficial mutants.

Keywords: *Tomato, Colchicine, Paradichlorobenzene*

INTRODUCTION

Tomato (Solanum lycopersicum, syn & Lycopersicon esculentum) is herbaceous, usually sprawling plant in the Solanaceae or night shade family, as its close cousins are tobacco, chilli peppers, and egg plant (Smithsonianmag, 2008). Tomato is often grown outdoors in temperate climates as an annual plant, typically reaching up to 1-3m (3 to 10ft) in height, with a weak, woody stem that often vines over other plants. The leaves are 10 - 25cm (3.9 - 9.8 inches) long, pinnate with 5 - 9 leaflets on the petioles (Acquuah, 2002). Each leaflet up to 8cm (3.1 inches) long, with a serrated margin; both the stem and leaves are densely glandular hairy. The flowers are 1 - 2cm (0.39 -0.79 inches) across, yellow, with five pointed lobes on the corolla. They are borne in cymes of 3 -12cm together (Krishna, 2007). Their flowers, appearing on the apical meristem have the anthers fused along the edges, forming a column surrounded by the pistil's style. Flowers tend to be self-fertilizing while others are pollinated by flies, butterflies, moths, other insects, or by other external forces. Tomato

fruit is classified as berry as well as a true fruit. It develops from the ovary of the plant after fertilization, its flesh comprises the pericarp walls. The fruit contains hollow spaces full of seeds and moisture called locular cavities (Krishna, 2007). Mutation is a sudden random change in the genetic material of a cell that may cause it and all cells derived from it to differ in some manner from the normal type. Mutations, either spontaneous or artificially induced both in seeds and vegetative propagated crops are of scientific and commercial interest. Mutations are the tools that Geneticists use in studying the nature and function of genes which are the building blocks and basis of plant growth and development, thus producing raw materials for genetic improvement of economic crops (Adamu, Chung and Abubakar, 2004). Various mutagenic agents are used to induce favorable mutations at high frequency. However, the use of ionizing radiations, such as x-rays and gamma rays, as well as chemical mutagens are well established (Ahloowalia and Maluszynski, 2001). Possibility of isolating beneficial mutants increased when higher frequency and wider spectrum of mutations are induced. This can be altered by various methods, such as post-treatment storage, combining different mutagens and applying mutagens at the particular time of the cell cycle (Nilan, 1973; Khalitkar and Bhatia, 1975). Mutants facilitate the isolation, identification and cloning of genes, which could ultimately help in designing crops with improved yield, increased stress tolerance, longer shelf life and reduced agronomic inputs (Ahloowalia and Maluszynski, 2001). Therefore, the aim of this work is to test the effects of the mutagens as a means of generating variations and useful traits in tomatoes.

MATERIALS AND METHODS

Matured seeds of Roma VF were obtained from Plant Science Department of the Institute of Agricultural Research (I. A. R.), Ahmadu Bello University, Zaria in Nigeria.

Mutagenic Treatment : ***First method (Seed treatment method):*** Matured air-dried seeds of Roma VF were pre-soaked in distilled water for five hours after which they were removed and properly dried. Equal seeds numbers of the Roma VF were then soaked in 1mm, 2mm, 3mm, and 4mm of Colchicine and also in 1mm, 2mm, 3mm, and 4mm of Paradichlorobenzene and 0.0mm (control), for both mutagens respectively. The treated seeds were then rinsed thoroughly with distilled water, partly dried and sown in boxes on vermiculite.

Second method: Seedling treatment method: The seeds were sown without being treated with any chemical but was watered daily at seedling level with the two chemical mutagens mentioned above at same concentration for 2 - 3 days.

Sowing of Seeds: ***First method:*** After the post-treatment washing, for the first method, the seeds were partially air dried and then planted in boxes on vermiculite. The seedlings were transplanted into planting bags, four weeks after germination. Each treatment was replicated five times following completely randomized design.

Second method: The seeds were transplanted after four weeks of germination, after which they have been watered with the chemicals as stated above. Each treatment

was replicated five times following completely randomized design. The data obtained for the study were statistically analysed using simple percentage, Mean rates and mean square estimated techniques.

RESULTS AND DISCUSSION

The results of the study indicate highly significance difference for germination percentage, number of leaves and height at maturity on tomato exposed to Colchicines (Table 1). There was decrease in germination percentage, seedling height and number of branches with increase in Colchicine concentration, and increase in leaf length, height at maturity with increase in Colchicine concentration for seed treatment method. However, there was a decrease in germination percentage, number of leaves, and height at maturity, while there was increase in seedling height, leaf length and number of branches for the seedling treatment method (Table 2).

Furthermore, results obtained indicated significant difference in the mean rates of effect of the Colchicine and seedling treatment shows the highest response to the chemical mutagen compared with seed treatment (Table 3). However, there was significance difference in seedling height, leaf length, number of leaves and height at maturity and highly significance difference in germination percentage. More so, the treatment indicates highly significance difference in germination percentage and significance difference in seedling height and number of branches. Similarly, the type showed highly significance difference in germination percentage, number of leaves and height at maturity and thus significance difference in leaf length. The interaction between treatment and concentration indicates highly significance difference only in germination percentage and significance difference in number of branches. However, interaction between concentration and type shows highly significance difference in germination percentage, seedling height, leaf length and significance difference in number of leaves. Furthermore interaction between treatment and type shows significance difference in seedling height, leaf length and height at maturity and highly significance difference in the germination percentage. In addition to which interaction between treatment, concentration, and type showed highly significance in germination percentage, and significance difference in leaf length and height at maturity (Table 4).

There was general decrease in all the traits studied with increase in concentration of Paradichlorobenzene for the seed treatment method. While there was decrease in germination percentage, number of leaves and height at maturity with increase in concentration and increase in seedling height, leaf length and number of branches with increase in concentration (Table 5). Furthermore, results obtained indicates significance difference in the mean rate of effect of Paradichlorobenzene on tomato and seedling treatment showed the highest response to the chemical compared with seed treatment (Table 6).

Results obtained from this study further show that all traits were affected significantly by treatment with either Colchicine or Paradichlorobenzene, in tomato

Roma VF. There was decrease in germination percentage, number of leaves, seedling height, number of branches, with increase in concentration of Colchicine and Paradichlorobenzene. This was in conformity with the work of Mensah and Obadoni (2007) who reported that, there were dose related effects of mutagenic treatment on qualitative traits resulting in reductions in traits such as germination and survival percentages, plant height, number of fruit per plant, and the findings of Adamu (2004) where groundnut was treated with gamma rays.

This was also in conformity with the findings of Adamu (2004) who reported that there was decrease in seedling height, root length of popcorn (Zea mays var praecox) with increase in gamma rays and thermal neutrons radiation. Furthermore, the research of Rauts, Jain and Gupta (1971) on cotton, Adamu (1997) reported on Zea mays var praecox, Takayi and Rahman (1995) reports on Soya beans (Glycine max), reveals that the number of leaves per seedling , number of pod per plant, number of branches per plant or seedling were dose dependent. This is possibly due to the mutation effects on the genetic composition of the Tomato leading to negative morphological traits. The height at maturity, number of branches per plant and number of fruits are not dose dependent. Abieu (1988) has reported in line with good agronomic characteristics that were phenotypically stable for height, yield and vigour in Maize. Also, Sasi, Dhanayel and Pavadai (2005) reported that all plant type mutants registered lower yield when compared to their parent Okra.

Table 1: Mean square estimate effect of Colchicine on Tomato (Lycopersicon esculentum)

Source of variation	Df	Germination % @ 5days	Germination % @ 8days	Seedling height(cm)	Leaf length (cm)	Number of leaves	Number of branches	Height at maturity (cm)
Treatment	1	1047.91**	588.45ns	0.77ns	0.13ns	89.78**	1.28ns	2760.25**
Error	48	67.27	181.19	1.45	0.09	1.82		67.23
Total	50							

** Significant difference (p<0.05) ** Highly significant difference (p<0.001), ns Non significant.*

Table 2: Effect of Colchicine on Tomato (Lycopersicon esculentum) growth parameters.

Type	Treatment	Germination % at 5days	Germination % at 8days	Seedling height(cm)	Leaf length (cm)	Number of leaves	Number of branches	Height at maturity (cm)
1	0Mm	26.43a	66.11a	7.14a	1.70b	4.00a	7.60a	39.60c
	1Mm	21.58c	62.91b	6.41b	2.01a	4.00a	7.20a	48.80ab
	2Mm	14.51d	36.86d	6.43b	2.10a	4.00a	7.50a	50.65a
	3mM	11.77e	42.56c	7.52c	2.45ab	4.00a	6.90b	49.50a
	4mM	22.18b	30.13e	6.54b	2.00a	4.00a	6.88b	44.33b
2	0mM	17.58d	38.95c	7.16a	1.96b	8.00a	6.70b	33.90c
	1mM	34.14a	54.26a	6.34b	1.90b	5.30ab	6.70b	38.90a
	2mM	25.56b	39.47c	6.72b	1.95b	6.60b	7.20a	35.00b
	3mM	21.15c	36.87d	5.59c	1.78ab	6.60b	6.70b	35.35b
	4mm	25.04b	43.85b	7.48a	2.15a	6.00b	7.36a	35.63b

Mean with the same letters within a column are not significantly difference.

Table 3: Mean rates of the effect of colchicine on Tomato (Lycopersicon esculentum)

Type	Treatment	Germination % at 5days	Germination % at 8days	Seedling height(cm)	Leaf length(cm)	Number of leaves	Number of branches	Height at maturity (cm)
1	Colchicine	14.90b	45.38a	6.34a	1.99a	4.00a	7.44a	48.88a
2	Colchicine	24.06a	38.69a	6.59a	2.09a	6.68b	7.12a	34.02b
	Mean +	19.48	42.04	6.47	2.05	5.34	7.28	41.45
	S.E -	1.32	1.94	0.16	0.04	0.27	0.16	1.56

Mean with the same letters within a column are not significantly difference.

Table 4: Mean square estimate effect of Colchicine and Paradichlorobenzene on Tomato (Lycopersicon esculentum)

Source of variation	Df	Germination % @ 5days	Germination % @ 8days	Seedling height(cm)	Leaf length (cm)	Number of leaves	Number of branches	Height at maturity (cm)
Concentration	4	359.94**	1846.63**	2.33*	0.26*	4.74*	0.89ns	168.08*
Treatment	1	648.21**	1091.64**	7.56*	0.19ns	0.49ns	4.00*	13.69ns
Type	1	757.35**	608.12**	0.48ns	0.29*	161.29**	1.96ns	3169.69**
Trt * Conc.	4	370.66**	606.47**	1.99ns	0.08ns	1.19ns	4.90*	32.18ns
Conc. * Type	4	382.65**	1160.92**	5.89**	0.64**	4.74*	1.31ns	75.65ns
Trt * Type	1	333.65**	3373.29**	3.72*	1.08*	0.49ns	0.04ns	324.00*
Trt*Conc.*Type	4	345.54**	729.95**	1.38ns	0.31*	1.19ns	1.94ns	141.78*

** Significant difference ($p<0.05$), ** Highly significant difference ($p<0.001$); ns Non significant difference.*

Table 5: Effect of Paradichlorobenzene on Tomato (Lycopersicon esculentum) growth parameters.

Type	Treatment	Germination % at 5days	Germination % at 8days	Seedling height(cm)	Leaf length (cm)	Number of leaves	Number of branches	Height at maturity (cm)
1	0Mm	26.57b	65.86b	7.14b	1.70b	4.00a	7.60a	39.60c
	1mM	36.08a	84.62a	6.98ab	1.98b	4.00a	6.80b	42.00d
	2mM	21.84c	45.48d	7.04b	2.20a	4.00a	7.00ab	49.30a
	3mM	11.64d	53.02c	8.58a	2.74a	4.00a	6.60b	46.00b
	4mM	22.37c	35.82e	6.65ab	1.90b	4.00a	7.00ab	44.50c
2	0mM	17.58e	30.20e	7.16a	1.96b	8.00a	6.80b	33.90c
	1mM	30.50b	45.72b	5.78b	1.62ab	4.60c	6.20b	42.40a
	2mM	35.60a	50.84a	6.96ab	1.82b	6.40b	5.80c	32.80d
	3mM	20.90d	32.94d	6.02ab	1.48ab	6.20b	7.20a	40.30b
	4mm	22.58c	40.81c	7.66a	2.13a	6.33b	7.66a	37.66c

Mean with the same letters within a column are not significantly difference

Table 6: Mean rates of the effect of Para-dichlorobenzene on Tomato (Lycopersicon esculentum)

Type	Treatment	Germination % at 5days	Germination % at 8days	Seedling height (cm)	Leaf length (cm)	Number of leaves	Number of branches	Height at maturity (cm)
1	Para-dichlorobenzene	25.49a	56.92a	7.28a	2.12a	4.00b	7.00a	44.54a
2	Para-dichlorobenzene	23.64a	40.37b	6.78a	1.80b	6.40a	6.76a	36.88b
	Mean +	24.57	48.65	7.02	1.96	5.20	6.88	40.71
	S.E -	1.06	2.23	0.14	0.06	0.22	0.14	1.09

Mean with the same letters within a column are not significantly difference

CONCLUSION

This work was to test the effects of the mutagens as a means of generating variations and useful traits in tomatoes. Colchicine and Paradichlorobenzene have both proved to be valuable agents in induction of beneficial and non beneficial mutations. The result showed that there were superior performances in 1mM concentration than the others including the control. 4mM showed better performance after the control in respect to effect on seedling height. Thus 1mM and 4mM concentrations of colchicines and Paradichlorobenzene can be used for induction of beneficial mutants in tomato (Lycopersicon esculentum). Therefore, the use of 1mM and 4mM Colchicine and Paradichlorobenzene is recommended for inducing mutation to create variability and thus increase the possibility of isolating beneficial mutants. Also there is need to conduct the test concurrently with other procedures such as test for disease resistance, test resistance or resistance to environmental stress in order to improve tomato production in Nigeria.

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