Induction of Genetic Variability in Pusa Jwala and Pusa Sadabahar (*Capsicum annuum* L.) through Mutation *via* Sodium Azide and Hydroxyl Amine

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Abstract

The present study was carried out to determine the mutagenic effect of Sodium Azide and hydroxyl amine on the morphological features of Pusa Jwala and Pusa Sadabahar (Capsicum annuum L.) in M_2 generation. Seed were pre-soaked in distilled water for 6hrs and later treated with different concentration w/v (0.1, 0.15, 0.20, 0.25) of sodium azide and hydroxyl amine mutagens for 6hrs. The experiment was planted Randomized block design, replicated three times. Observation on growth parameters of M_2 mutants of Pusa Jwala and Pusa Sadabahar shown significant variation on the plant height, germination, primary secondary branches, length of fruit, test weight of seed, number of fruits per plant, days to first flower mortality and survival. Most of the parameters decreased with increase in concentration of sodium azide. The result of study shown that sodium azide significantly affects plant height, germination and days to first flowering. Sodium Azide is more effective as compared to the Hydroxyl Amine in inducing genetic variability.

Keywords: Chilli, Mutant, Sodium Azide, Hydroxyl Amine, Pusa Jwala, Pusa Sadabahar, Variability

Introduction

Chilli (*Capsicum annuum L.*) is a spice cum vegetable crop belongs to the family Solanaceae. The plant is self-pollinated with 2n=24 chromosomes and its Centre of origin is Mexico. (Pickersgill, 1991 Shobha. B, 2016, Moscone *et al.*, 2007). India contributes in 2020-21 world chilli production around 19.88 (lakh tonnes) it means 36.57 per cent and productivity is 2716 (kg/ha) (AMIC, PJTSAU JAN 2022). Chilli contributes for 33 Per cent of world spices trade in terms of volume and India is major producer, consumer and exporter in the world. Chilli accounts for 31-38 Per cent of total Indian spices exports. Chilli consumed as green vegetable, chilli paste and it contain steam-volatile oil, fatty oils, capsaicinoids, carotenoids, vitamins (C, A and E) (Votava and Tomas 2003, Andres, 2013), protein, fibre, and minerals (Abu *et al.*, 2019 b). It has high commercial value and medicinal values like anti cancerous and antioxidant properties. Induced mutations can rapidly create variability in quantitatively and qualitatively inherited traits in crops. Now-a-days, mutation breeding is used as an additional tool by the plant breeders. Mutation is a sudden heritable change, brought out in a single nucleotide base pair either by addition, deletion or

substitution caused by the various factors which leads to a change in the coded information finally expressed in terms of changed phenotypes through alteration in the chain of events like transcription and translation. Mutation provides the variability and a basic raw material for the plant breeder and it is very useful to inducing the new variability, which is an essential requirement of any plant breeding program, for changing pattern of today's agriculture.

Materials and Method

The study for the present investigation was conducted from June-December 2020 in the Crop Research Centre of Department (CRC), Department of Genetics and Plant Breeding, School of Agriculture, ITM University, Gwalior, M.P., India. The seeds of Pusa Jwala and Pusa Sadabahar (*Capsicum annuum L*.) were subjected to different treatment levels of mutagen *viz*. Sodium Azide (NaN_3) and Hydroxyl Amine (H_3NO). Treatment parameters were four different concentration (0-control) 0.1, 0.15, 0.20, 0.25 weight by volume. The solution of mutagen was prepared by dissolving 0.1, 0.15, 0.20, 0.25 gram of sodium azide and hydroxylamine in 100 ml of distil water respectively, and second to form 0.1, 0.15, 0.2, 0.25% weight by volume concentration of sodium azide and hydroxyl Amine in 100 ml of distilled water, respectively and shaken to form 0.1, 0.15, 0.20, 0.25 per cent weight by volume concentration of Sodium Azide and Hydroxyl Amine respectively.

Before treatment seeds were pre-soaked in five different petri dishes containing distilled water for six hours at room temperature to enhance the effect of mutagen. There after five group of Pusa Jwala and Pusa Sadabahar were subject to 4 (four) concentration (0.1, 0.15, 0.2, 0.25) of Sodium azide (NaN_3) and hydroxyl amine (H_3NO) solution and the control at room temperature 25°C for six hours. After treatment the seeds washed thoroughly with distilled water to remove the residual mutagens and air dried on filter paper. 100 seeds of each treatment were sown immediately in germination basket containing amended soil (Top soil: poultry droppings: run-off soil in ratio of 3:2:1) (Ojua *et.al.*, 2019). Germination of the seed started in about 10 to 15 days. Nursery was ready after 45 days of sowing. Each plant was transplanted in the field containing 20 per cent of the amended soil. The treated seedlings were laid out using Randomized Block Design (RBD) with three replications. plants were properly irrigated and all the cultural practices were timely performed to evaluate the morphological and yield trait of Pusa Jwala and Pusa Sadabahar with sodium azide and hydroxyl amine mutagen. The morphological trait studied include; Germination percentage, number of primary branches and secondary branches, days to first flowering, length of fruit, number of fruits per plant, yield per plot, mortality percentage, survival and seed test weight.

Data collected were subjected to Analysis of Variance using OP STAT (O.P. Sheoran Programmer, Computer Section, CCS HAU, Hissar) and significant mean were separated using t-test (One factor analysis). 25 plants were selected on the basis of significant change in the characters germination percentage, plant height and days to first flower. These selected plants were expected to be mutant for which the progeny generation of this plants were raised in the next year (June-Dec 2021). The number of plants raised in M_2 generation were 75 in three replications for each suspected mutant from M_1. The M_2 generation was planted with plan to plant and row to row distance as 60 x 30 cm. Single plant per hill was raised to record the data properly. All the field practices were maintained as per the requirement to raise a good crop.

Result and discussion

Mutants were characterized on morphological basis of plant type. Over all 25 mutants where isolated, the central matter in this mutant analysis concern in the viable mutant may of which weather, they are morphological in characters have potential value in plant breeding a considerable rate of mutants were obtained with the mutagen sodium azide

and hydrogen amine. The frequency of mutant with the desirable viable morphological plant is present in the Table 1 (a)(b) and Table 2 (a)(b).

Mutants in the M_1 generation were characterized with respect to the characters, plant height, germination, days to first flowering. The characteristic of the identified mutants is discussed under the following heads.

Table 1. (a) Analysis of variance of Pusa Jwala

Treatment	Plant Height (cm)		No. of Primary Branches		No. of Secondary Branches		Germination Percentage (%)		Days to first Flowering		No. of Fruit per plant	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1	74.24	0.04	4.53	0.16	6.33	0.13	78.67	0.34	37.80	0.00	185.11	0.28
2	72.26	0.08	4.13	0.16	5.80	0.00	76.11	0.22	38.87	0.17	183.11	0.11
3	71.34	0.01	4.00	0.15	5.67	0.07	75.11	0.10	39.33	0.06	180.77	0.10
4	70.32	0.08	3.73	0.07	5.20	0.36	72.78	0.10	40.40	0.15	178.77	0.10
5	73.90	0.44	4.53	0.13	6.40	0.15	80.33	0.00	37.80	0.00	186.33	0.19
6	72.32	0.02	3.93	0.07	5.73	0.07	78.00	0.12	39.00	0.20	184.77	0.10
7	70.26	0.00	3.73	0.07	5.27	0.21	72.78	0.22	40.20	0.00	181.22	0.11
8	68.22	0.00	3.47	0.07	4.27	0.07	69.00	0.12	40.60	0.305	174.66	0.19
9	76.94	0.67	5.13	0.16	6.80	0.00	84.22	0.19	35.80	0.23	200.88	0.48
C.D.	0.91		0.25		0.47		0.53		0.34		0.64	
SE(m)	0.30		0.04		0.14		0.16		0.10		0.21	
SE(d)	0.42		0.13		0.24		0.23		0.10		0.30	
C.V.	0.72		3.91		4.35		0.42		0.56		0.20	

Table 1. (b) Analysis of variance of Pusa Jwala

Treatment	Length of Fruit (cm)		Seed Test Weight (g)		Green Fruit yield per plant (kg)		Survival (%)		Mortality (%)	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1	9.58	0.05	4.61	0.00	3.81	0.00	88.89	2.22	11.10	2.22
2	9.51	0.02	4.58	0.00	3.76	0.00	84.44	2.22	15.55	2.22
3	9.51	0.01	4.59	0.00	3.63	0.00	82.22	2.22	17.77	2.22
4	8.89	0.01	4.57	0.00	3.72	0.00	80.00	3.84	19.99	3.84
5	9.62	0.01	4.62	0.00	3.88	0.00	91.11	2.22	8.88	2.22
6	9.17	0.02	4.60	0.00	3.71	0.00	88.89	2.22	11.10	2.22
7	8.67	0.01	4.57	0.00	3.53	0.00	82.22	2.22	17.77	2.22
8	8.18	0.01	4.52	0.00	3.25	0.00	77.78	2.22	22.22	2.22
9	9.33	0.00	4.65	0.00	4.30	0.00	93.34	0.00	6.66	0.00
C.D.	0.06		0.00		0.00		5.76		5.76	
SE(m)	0.02		0.00		0.00		1.90		1.90	
SE(d)	0.03		0.00		0.00		2.69		2.69	
C.V.	0.42		0.09		0.11		3.86		22.67	

Table 2. (a) Analysis of variance Pusa Sadabahar

Treatment V2	Plant Height (cm)		No. of Primary Branches		No. of Secondary Branches		Germination Percentage (%)		Days to first Flowering		No. of Fruit per plant	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1	52.36	0.01	4.46	0.17	6.26	0.06	77.77	0.11	41.73	0.24	77.00	0.19
2	51.28	0.02	4.06	0.17	5.66	0.13	75.44	0.10	43.06	0.26	75.33	0.19
3	50.26	0.04	3.80	0.00	5.73	0.06	73.55	0.29	43.60	0.41	72.44	0.10
4	49.38	0.02	3.60	0.00	5.86	0.29	70.44	0.40	44.00	0.41	71.11	0.22
5	53.43	0.16	4.73	0.06	6.33	0.13	77.88	0.11	42.06	0.13	80.44	0.10
6	51.29	0.05	4.00	0.11	6.00	0.30	75.66	0.00	42.80	0.00	76.44	0.10

7	49.66	0.35	3.80	0.00	5.40	0.30	71.11	0.11	44.33	0.13	70.55	0.22
8	48.00	0.22	3.40	0.11	4.53	0.52	65.44	0.22	45.06	0.37	65.55	0.11
9	56.18	0.12	5.33	0.06	6.66	0.06	77.55	0.11	39.26	0.29	90.77	0.22
C.D.	0.50		0.32		0.72		0.58		0.77		0.55	
SE(m)	0.16		0.10		0.23		0.19		0.25		0.18	
SE(d)	0.23		0.15		0.33		0.27		0.36		0.25	
C.V.	0.56		4.49		7.10		0.45		1.03		0.42	

Table 2. (b) Analysis of variance of Pusa Sadabahar

Treatment	Length of Fruit (cm)		Seed Test Weight (g)		Green Fruit yield per plant (kg)		Survival (%)		Mortality (%)	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1	6.14	0.01	4.55	0.00	2.81	0.00	88.89	2.22	11.10	2.22
2	6.02	0.01	4.52	0.00	2.76	0.00	84.44	2.22	15.55	2.22
3	5.88	0.01	4.51	0.00	2.71	0.00	82.22	2.22	17.77	2.22
4	5.81	0.01	4.48	0.00	2.63	0.01	77.78	2.22	22.22	2.22
5	6.14	0.01	4.55	0.00	2.85	0.00	91.11	2.22	8.88	2.22
6	6.04	0.01	4.51	0.00	2.74	0.00	88.89	2.22	11.10	2.22
7	5.85	0.01	4.47	0.00	2.33	0.00	77.78	2.22	22.22	2.22
8	5.05	0.02	4.40	0.00	2.10	0.00	77.78	2.22	22.22	2.22
9	6.31	0.01	4.71	0.00	3.22	0.00	93.34	0.00	6.66	0.00
C.D.	0.04		0.00		0.02		6.08		6.08	
SE(m)	0.01		0.00		0.00		2.01		2.01	
SE(d)	0.01		0.00		0.01		2.84		2.84	
C.V.	0.38		0.10		0.43		4.11		22.76	

Germination

The dose of sodium azide with the concentration of 0.20% was found to be most effective in variety Pusa Sadabahar on contrary to the control (78%). The M_1 and M_2 generation was investigated with a significant reduction in germination that is 70 and 68 per cent respectively, (Fig 1). The drastic reduction in the germination with increase in the concentration of sodium azide and hydroxyl amine may be due to induction of chromosomal structural abnormalities in the genes of vitality. This result is in synchronisation with Motilal *et al.,* (2012) Ojua *et al.,* (2019).

Plant height

The field performance of M_1 generation revealed significant variation in plant height. The control of both the varieties Pusa Jwala and Pusa Sadabahar displayed 78.8 cm and 55.1 cm respectively. Both the varieties displayed significant variation in the M_1 generation for plant height. The effective dose of mutagen was found to be 0.1% in Pusa Jwala and 0.25% in Pusa sadabahar with the mutagen sodium azide. The M_1 generation of Pusa Jwala had a plant height of 67.3 cm. similarly, In M_2 generation the plant height observed to be 68.0 cm in case of treatment with 0.1% sodium azide. Pusa Jwala also displayed the same trend with a significant reduction in height *i.e*, 44.2 cm and 45.6 cm in M_1 and M_2 generation respectively (Fig 1). The result here may be due to the effect of chemical mutagen which supress the early development in plant. This result is in agreement with (Ashish establishment 2011, Jagannath 2013, omitted 2021)

Days to first flowering

An increase in days to first flower an evident in the variety Pusa Jwala. The most effective dose for this alteration was experimented to be 0.20% in sodium azide. which leads to 42 days in M_1 and 46 days in M_2 as well (Fig 1). Due to the presence of suspected chromosomal aberration a delay in flowering was also reported by Patil (2009) in cow pea plant

and Lal *et al.*, (2009) in chilli and Ojua *et al.*, (2019).

Table 3. Comparison of Control, M_1 and M_2

Character	Mutagen	Dose	Variety	Control	M1	M2
Germination (%)	S.A	0.20	Pusa Sadabahar	78	70	68
Plant height (cm)	S.A	0.10	Pusa Jwala	78.8	63.3	68.0
Plant height (cm)	S.A	0.25	Pusa Sadabahar	55.1	44.2	45.6
Days to first flowering	S.A	0.20	Pusa Jwala	35	42	46

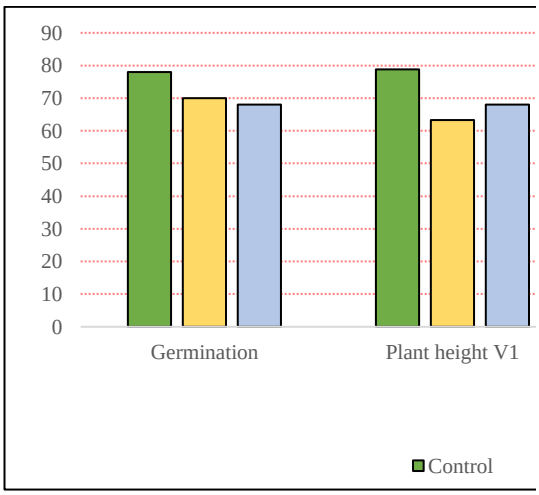

Fig 1. Comparative representation of control, M_1 and M_2

Conclusion

The variation in the morphological features in the M1 mutant, Pusa Jwala is an indication that sodium azide is more effective as compared to the hydroxyl amine in inducing genetic variability in the quantitative trades generally, differences in concentration of the mutagen significantly affected most of the parameters evaluated in the given varieties. The result of study shown that sodium azide significant effect on plant height, germination and days to first flowering. Therefore, induce mutation is highly effective in creating variability of desirable trade in Chilli for further reading program.

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