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INDUCTION OF MUTATIONS IN *VIGNA RADIATA* L.WILCZEK USING HYPOXANTHINE, A PURINE BASE ANALOGUE

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Several types of morphological and chlorophyll mutants were isolated from the M_2 and M_3 segregating families of *Vigna radiata* L. Wilczek var. K-851 after treatment with different doses of hypoxanthine, a purine base analogue. The results obtained clearly indicate that hypoxanthine has the potentiality to act as a mutagen in higher plants.

Key words : Hypoxanthine, base analogue, *Vigna radiata*, mutants, genetical analysis.

In the advanced breeding programmes, mutations are induced by various chemical and physical mutagens. In the past various physical and chemical mutagens have been widely used to induce mutations in *Vigna radita* by various workers (Raghuvanshi *et al.*, 1978; Grover and Tejpaul, 1980; Singh and Chaturvedi, 1981; Thakare *et al.*, 1983; Singh and Yadav, 1991). The mutagenic activity of 5-azacytidine (5-Azt) (a pyrimidine base analogue) on *Escherichia coli* has been tested by Fucik *et al.* (1965). So far the mutagenicity of hypoxanthine, a purine base analogue, on higher plants or on bacterial test system has not yet been tested by any investigator. Therefore, an attempt has been made to study the mutagenic effects of hypoxanthine on mungbean, a common pulse crop.

MATERIALS AND METHODS

Dry pure line seeds of *Vigna radita* L. Wilczek var. K-851 were surface sterilized with 0.10% $HgCl_2$ and soaked in distilled water for 12h. The presoaked seeds were then treated with freshly prepared solutions of hypoxanthine (0.0003%, 0.0005%, 0.0010%, 0.0050%, 0.0100%) for 24h. (300-500 seeds for each concentration) with intermittent shaking. After thorough washing, treated seeds alongwith control ones were sown immediately in the field to raise the M_1 generation.

To isolate and observe the segregation pattern of mutants in M_2 populations, 20-30 M_2 segregating progenies were raised from the seeds of normal looking M_1 plants showing minimum phytotoxic effects as well as from the plants showing altered morphology. The M_2/M_3 segregant families were screened during vegetative, flowering and fruiting periods to isolate the mutants. Lines exhibiting only normal phenotypes were discarded, while from those segregating for mutant phenotype, a random selection of normal plants was done to collect seeds for M_3 hetrozygous lines. Genetical analysis of a few selected mutants particularly the inheritance of mutated trait(s) was studied from the segregation pattern of the heterozygous lines in M_2/M_3 generation.

RESULTS AND DISCUSSIONS

The frequency of morphological mutants including nodulating as well as chlorophyll mutants were recorded (Table 1) as mutantion per cent M_2 seedlings. From the segregating families, interesting type of habit, leaf, stem, flower, root and chlorophyll mutants were detected. The important altered characteristics of the mutants are summarised in the Table 2. Brief description of detected mutants is enumerated below:

Morphological Mutants

Habit Mutant : Tall

Increased plant height (37.59% increase over control) was the stricking feature of the mutants and could be easily identified at the early developmental stage. Mutation viable.

Habit mutant : - Dwarf

The mutant was recognised at seedling stage. The mutant plants were stunted and had small sized leaves and reduced internodal length. Mutation viable.

Leaf mutant : - Tricotyledonary leaf

Mutant had three cotyledonary leaves instead of the two in control. Mutation viable. (Fig. 1b).

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Table 1: Frequency of morphological and chlorophyll mutants in M_2 generation (Per cent M_2 plants)

Hypoxanthine series (%)	Habit mutants		Leaf mutants			Flower mutant	Root mutants			Chlorophyll mutants		
	Tall	Dwarf	Tricotyledonary leaf	Lobed lamina	Altered orientation	Exposed stigma	Fibrous root	High frequency of nodules	Low frequency of nodules	Lutina	Xanthina virescence	Virina xanthescence
Control	-	-	-	-	-	-	-	-	-	-	-	-
0.0003	2.56	0.51	-	4.10	0.51	-	1.02	0.51	-	-	0.51	-
0.0005	3.06	1.02	-	-	1.53	-	0.51	1.53	-	-	-	-
0.0010	-	0.70	-	-	0.70	-	-	1.40	-	-	-	2.11
0.0050	1.44	1.44	-	-	-	1.44	-	-	-	-	1.44	-
0.0100	-	-	2.49	-	1.21	-	-	-	3.65	0.72	-	3.65

Leaf mutant : - Lobed lamina

Mature leaf with lobed lamina was the stricking feature recorded in this mutant. Mutation viable. (Fig. 1c).

Leaf mutant : - Altered orientation

The first and second leaves formed on the mutant plants were opposite to each other, unlike the alternate pattern in control. Mutation viable. (Fig. 1d).

Flower mutant : - Exposed stigma

As compared to control, flowers with long style, exposed stigma (stigma protruding out of the flowers) and small sized stamens were the distinguished features of the mutant plants. Mutation non-viable.

Root mutant : - Fibrous root

Instead of tap root, as observed in mother variety, fibrous root was observed in the mutant plants. The mutated trait was associated with increased seed yield, number of lateral roots and number of nodules, Mutation viable. (Fig. 2b.)

Table 2: Comparison of induced mutants of *V. radiata* L. with mother variety for different traits.

Control/Mutants	Plant height (cm) mean+SE	No. of flowers/ plant mean+SE	No. of pods/ plant mean+SE	No. of seeds/ plant mean+SE	Root length (cm) mean+SE	No. of lateral roots/ plant mean+SE	No. of nodules/ plant Mean +SE
Control	54.0±0.32	38.0±0.42	25.5±0.60	201.3±0.57	27.7±0.62	36.2±0.53	26.0±0.34
Habit mutants							
Tall	74.3±0.48	40.2±0.37	28.0±0.75	308.0±0.41	25.0±0.38	42.1±0.35	35.2±0.43
Dwarf	27.8±0.37	17.5±0.40	10.7±0.56	69.7±0.63	14.2±0.27	18.2±0.57	17.0±0.35
Leaf mutants							
Tricotyledonary	57.0±0.53	40.2±0.39	27.3±0.48	202.5±0.35	29.7±0.63	39.0±0.49	30.8±0.51
Lobed lamina	52.0±0.47	43.0±0.51	30.7±0.42	236.0±0.50	29.5±0.71	40.0±0.51	32.1±0.46
Altered orientation	64.2±0.23	45.3±0.48	29.6±0.57	227.5±0.42	32.0±0.50	37.0±0.74	30.7±0.67
Flower mutant							
Exposed stigma	48.1±0.38	10.4±0.70	-	-	18.2±0.51	27.2±0.40	17.0±0.72
Root mutants							
Fibrous root	62.0±0.55	44.0±0.56	37.0±0.52	248.5±0.43	28.7±0.35	78.0±0.54	52.3±0.43
High frequency of nodules	53.0±0.38	49.0±0.43	35.0±0.47	247.6±0.38	37.2±0.54	50.6±0.42	69.6±0.45
Low frequency of nodules	34.0±0.19	20.7±0.39	14.0±0.52	79.0±0.81	19.5±0.32	21.7±0.38	14.0±0.75
Chlorophyll mutants							
Lutina	19.0±0.37	-	-	-	-10.3±0.42	14.0±0.57	10.7±0.35
Xanthina-virescence	37.0±0.52	18.7±0.71	10.3±0.54	76.0±0.67	15.7±0.39	26.5±0.51	18.5±0.42
Virina-xanthescence	32.0±0.39	-	-	-	16.4±0.42	19.5±0.74	13.4±0.57

Table 3: Segregation ratio of mutants in heterozygous lines in M\ generation of *V. radiata* L.

Mutant types	No. of families studied	No. of families segregating	Segregation in families				
			Normal phenotype	Mutant phenotype	Assumed segregation	X^2	p%
Habit mutants							
Tall	7	3	73	23	3:1	0.223	50-75
Dwarf	11	2	67	20	3:1	0.188	50-75
Leaf mutants							
Tricotyledonary	10	2	72	20	3:1	0.522	25-50
Altered orientation	5	2	49	17	3:1	0.281	50-75
Root mutants							
Fibrous root	13	2	43	5	8:1	(Abnormal)	-
High frequency of nodules	11	2	83	11	8:1	(Abnormal)	-
Low frequency of nodules	10	1	37	11	3:1	0.256	50-75
Chlorophyll mutants							
Lutina	7	1	38	3	13:1	(Abnormal)	-
Xanthina-virescence	12	2	35	9	3:1	0.485	25-50
Virina-xanthescence	10	1	28	8	3:1	0.148	50-75

Root mutant :- Higher frequency of nodules

Increased frequency of nodules (167.69% increase over control) and lateral roots (39.77% over control) was recorded. Mutation viable. (Fig. 2c).

Root mutant :- Low frequency of nodules

Reduced frequency (46.15% as compared to control) of nodules was observed in the mutant plants. Reduced root length and number of lateral roots were the other characteristic alterations observed in mutant plants. Mutation viable. (Fig. 2d).

Chlorophyll Mutants

Lutina

Cotyledonary leaves formed on mutant seedlings were golden yellow in colour throughout the surviving period (5-10 days). In another category of lutina, the mutant plant had normal cotyledonary leaves but mature leaves were golden yellow. Plant survived for a period of 30-35 days. Mutation lethal. (Fig. 1e).

Xanthina - virescence

The emerged young leaves were light yellow in colour. However, later, yellow colour of the leaves turned into normal green within a period of 7-10 days. Mutation viable. (Fig. 1f).

Virina-xanthescence

After 20-25 days of growth and development, normal green colour of the mature leaf turned into yellow. The decoloration of the leaves would start either from the tip, base or from the margins of the lamina. Muation lethal. (Fig. 1g).

Isolated viable mutants exhibited true breeding behaviour in M_3 generation.

In the past, base analogue has been used for their antitumour and other biological activities. According to Doerfler (1983) treatment of base analogues especially 5-Azt has produced new pattern of differentiation in eukaryotic cell culture. Fucik *et al.* (1965) were of the opinion that the inhibitory effects of 5-Azt is accompanied by chromosome stickness and aberrations in plants. However, the mutagenic effectiveness of hypoxanthine in higher plants has not been demonstrated. The present observation have clearly revealed that hypoxanthine could induce useful mutations. This is supported by the good number of mutations produced by this analogue. Amongst the viable mutants, habit (tall), leaf (lobed lamina, altered orientation) and root mutants (higher frequency of nodules and fibrous root) showed improved vegetative and reproductive characters when compared with mother variety. In addition, hypoxanthine could also produce a few non-viable mutants. Mutants like exposed stigma flower, virina-xanthescence and lutina showed complete sterility or lethality in homozygous state.

Isolation of root mutants in the mutation research programme has been completely neglected (Gottschalk & Wolff, 1983) Limited number of root mutants has been reported in various crop plants (Gorney, 1978;

Quedanau, 1972). Another stricking result obtained in the present study was the isolation of fibrous root mutant with increased productivity and nodulation.

Segregation pattern of majority of the isolated mutants in hetrozygous M_2/M_3 lines has been used to asses the probable genetical status of the mutants as well as for knowing the genetical structure of the M_1 plants raised from mutagenized seeds. Nevertheless, variants recorded in M_2 were confirmed as mutants after the altered trait(s) reappeared in subsequent generations. Analysis of segregation pattern of M_2 heterozygous lines indicated, relative to the normal phenotype, less number of mutant phenotype. However, the progenies of the plant in M_3 for the mutant allels, tall, dwarf, tricotyledonary leaf, altered orientation (leaf), low frequency of nodules, xanthina-virescence and virina-xanthescence showed a 3:1 segregation ratio (Table 3) indicating monogenic recessive inheritance of the traits and origin of these mutants fsom genetical uniform M1 (no cheimeric structure) treated plants. However, the progenies of plants heterozygous in M_3 for the mutant alleles, high frequency of nodules, fibrous root and lutina did not show a monohybrid segregation ratio. Genetics of aforesaid three mutants is not clearly understood. Nevertheless, as per Gottschalk and Wolff (1983) when out of many embryonic initial cells, one of them mutates and rest survives and unaffected cells then unfavourable segregation ratios as observed for high frequency of nodules (8:1), fibrous root (8:1), lutina (13:1), mutants are formed indicating chiemerical nature of the mutagenized M1 plants.

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