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Induced High Punicic Acid Yielding Mutants of *Trichosanthes anguina*

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High punicic acid-yielding mutants have been isolated from X-irradiated and colchicine treated populations of two varieties of *Trichosanthes anguina*. The mutants yielded 11% to 32% increase in punicic acid over their respective parent line.

Key Words - *Trichosanthes* X-rays Colchicine Mutation Punicic acid

***Trichosanthes anguina* L. belonging to the family Cucurbitaceae is cultivated throughout India, particularly in the plains of Eastern India, for its fruits which are used as a summer vegetable. Its seeds yield drying oils (Hopkins & Chisholm 1962). One of the essential constituents of this oil is punicic acid, which is an isomer of L-eleostearic acid of tung oil (Ahlers & Dennison 1954, Toyama & Tsuchiya 1953, Saha 1974). Seed oil, like that obtained from *T. anguina*, which contains large proportion of eleostearic acid is highly useful in paint industry, in the manufacture of protective coatings, especially in quick-drying, oleoresinous varnishes and enamels (Hoffmann *et al.*, 1957, Hilditch 1956). This plant, although has both agricultural and industrial importance has not received crop improvement programme.**

Two varieties of *T. anguina*, were included in a crop improvement programme through X-ray and colchicine induced mutations with a view to isolate mutants with high punicic acid content in the seed oil. This paper deals with the high punicic acid yielding mutants of *T. anguina*.

MATERIALS & METHODS Two fruit colour varieties, namely, White (white stripe on white background) and Green-White Stripe (white stripe on green background) of *T. anguina* were used.

The moisture content of dry seeds was measured before treatment with X-rays and colchicine in a Torsion Balance Moisture Meter (Associated Instruments, Calcutta). Dried seeds were treated with 6, 12, 18, 24 and 30 kR X-rays from a Philips Contact and Cavity Therapy Tube. Dried seeds were kept immersed in 0.25, 0,50 and 1.00% aqueous solutions of colchicine for 18 h. Control seeds were kept immersed in distilled water for the same period. The treated and control seeds were washed in running water for 3 h before sowing.

To measure seed oil and punicic acid in the oil, seeds were weighed and crushed in mortar and pestle in presence of anhydrous sodium sulphate. Petroleum ether (40°-60°C) 5 mL was added and stirred. The seed extract was decanted and filtered into a volumetric flask. This process was repeated 4 times. The final stock solution was made up to 25 mL with petroleum ether. A 5 mL aliquot of the stock solution was transferred to a tared 5 mL volumetric flask containing a few porcelain beads. The solvent was evaporated in a vacuum desicator under reduced pressure. The flask was weighed again. The difference in weight yielded the total oil in 5 mL of stock solution. An aliquot of the stock solution was diluted with cyclohexane (special for spectroscopy) so that the concentration of the final solution was in the range of 0.006-0.007 g/L. The optical density of the final solution was measured at 275 nm in a Beckman DU spectrophotometer. The amount of punicic acid present in the seed oil was calculated (Hopkins & Chisholm 1962).

In the White and Green-White Stripe variety, the X-irradiated and colchicine treated plants were grown bulk treatment wise in the second (M_2 and C_2) generation. Single plant selections for high punicic acid content in the seed oil in each population were made in the second generation and studied separately. The selected plants were grown after self fertilization by artificial pollination to raise subsequent generations.

Selections from the treated populations which showed little or no difference in mean punicic acid content from that of the control or had low punicic acid content in the third generation were generally discarded. Single plant selections based on higher punicic acid content than that of the control were repeated in the third generation and studied in the fourth generation.

RESULTS & DISCUSSION **The control populations of the White and Green-White Stripe varieties of *T. anguina* are true breeding with reference to punicic acid content. However, two selections each from the control populations of two varieties were made. In the White variety WP1 and WP2 represented the two selections from the control populations. Four selections (WP3-WP6) from 6kR, four selections (WP7-WP10) from 24kR, three (WP11-WP13) from 30kR and four (WP14-WP17) from 0.50% colchicine treated populations were made. Similarly two (GWS1 and GWSP2) from control, two (GWSP3 and GWSP4) from 12kR, three (GWSP5-GWSP7) from 18 kR, two (GWSP8 and GWSP9) from 30kR, two (GWSP10 and GWSP11) from 0.25% and three (GWSP12-GWSP14) from 0.50% colchicine treated populations were made in the cultivar Green-White Stripe.**

Out of 17 selections in the White cultivar, selections WP3, WP7, WP8, WP12, WP14 and WP16 showed significant increase in punicic acid content over the control in the third generation. All the selections except WP7 continued to show more significant increase in punicic acid content than the control in the fourth generation (Table 1). The experiment indicated that the control population was a true breeding material with respect to punicic acid content. An attempt was made to prove true breeding nature of the selections for high punicic acid yielding mutants. Two plants, one showing the lowest and the other showing highest punicic acid content of each of WP3, WP8, WP12, WP14 and WP16 were selected in the third generation and studied in the fourth generation. WP3, WP8, WP12, WP14 and WP16 were true breeding with respect to punicic acid content (Table 1). It was, therefore, possible to isolate 5 true breeding high punicic acid yielding mutants in the White cultivar.

Table 1 Punicic acid in the Selected Lines in the 3rd and 4th Generations After Treatment with X-rays and Colchicine in the White and Green-White Stripe Cultivars of *T. anguina*

Selection	Generation 3rd	Generation 4th	Punicic acid(%) of the entire population of 4th generation	Punicic acid (as % of mother line)
WP1	54	55	55	100
		54		
WP3	61***	62***	67	112
		60***		
WP7	62***	60	58	
WP8	62***	60***	62	112
		63***		
WP12	60*	61*		
WP14	61***	59+	61	111
		62***		
WP16	65***	63***	64	116
		65***		
GWSP1	52	53	53	100
		52		
GWSP6	68***	67***	66	125
		65***		
GWSP7	72***	71***	70	132
		68***		

* = P∠0.05, + = ∠0.02, *** = P∠0.001

In the Green-White Stripe variety out of 14 selections made from different treated populations only two, GWSP6 and GWSP7 showed signi-

ficant increase in punicic acid content over the control in the third generation (Table 2). Like the White cultivar, two selections each from the control, and GWSP6 and GWSP7 were made in the third generation. The control populations of the Green-White Stripe variety bred true with respect to punicic acid content. GWSP6 and GWSP7 continued to show significantly higher punicic acid content than the mother line in the fourth generation. These two high punicic acid mutants were tested for pure line. They were true breeding mutants (Table 2).

Table 2 Punicic acid, total oil and seed weight, in the mother line and high yielding mutants in the White and Green - White Stripe cultivars of *T. anguina*

Selection	Punicic acid %	Total seed oil %	Seed weight (g)
WP1 (Mother line)	54	25	0.264
Mutant WP3	61***	26	0.283
WP7	62***	24	0.256
WP8	62***	28*	0.279
WP12	60*	23	0.264
WP14	61**	28*	0.277
WP16	65***	27**	0.293
GWSP1 (Mother line)	52	25	0.262
Mutant GWSP6	68***	28**	0.317
GWSP7	72***	27	0.272

* = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$

The five true breeding high punicic acid mutants of the White variety and two of the Green-White Stripe variety showed increase in punicic acid content in the seed oil by 1-16% and 25-32% respectively over their respective mother line (Tables 1 and 2).

Selections WP8, WP14 and WP16 had higher seed oil than the mother line. Seed weight in WP16 was also significantly high. In the Green-White Stripe variety, one of the mutants, GWSP6 surpassed its mother line in seed weight, percentage f oil and percentage of punicic acid in the seed oil. Total oil content also significantly increased in GWSP7. Two other mutants GWSP5 and GWSP14 had increased seed weight and percentage of seed oil over those of the mother line. Both X-ray and colchicine induced mutants are beneficial from industrial point of view as they yield high percentage of punicic acid.

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REFERENCES

AHLERS N H E & A C DENNISON 1954 The spectroscopic examination of snake gourd oils, *Chem Industry* 603.

HILDITCH T P 1956 The chemical constitution of natural fats, Chapman & Hall, London.

HOFFMANN J S, R T O'CONNOR, D C HEINZELMAN & W G BLACKFORD 1957 A simplified method for the preparation of L-eleostearic acids and revised spectrophotometric procedure for their determination, *J Am Oil Chem Soc* 7 338-342.

HOPKINS C Y & M J CHISHOLM 1962 Identification of conjugated triene fatty acid in certain seed oils, *Can J Chem* 40 2078-2082.

SAHA S 1974 Investigations on the fatty acids and glyceride composition of some less familiar seed oils, Ph. D, Thesis, Univ Calcutta, Calcutta.

TOYAMA Y & T TSUCHIYA 1953 Another new stereoisomer of eleostearic acid in the seed oil of Karasuuri (*Trichosanthes cucumeroids*), *J Soc Chem Ind Japan* 38 185-187.