ANALYSIS OF GENETIC ARCHITECTURE FOR PUNGENCY (PYRUVIC ACID) IN ONION (*ALLIUM CEPA* L.)

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ONION has a wide range of variability and very little work has been done to exploit it in breeding programmes. Hence a study was conducted on the gene action on pungency in onion through diallel analysis. The pyruvic acid is used as a measure of pungency, which is a very important character for the better storage qualities and processing. The 8 promosing inbred lines having distinct phenotypic diversities were crosses (table 1) and 28 F_1 hybrids along with 8 parents were evaluated in a randomized block design. A known amount of onion was peeled and the juice extracted. For the analysis of pyruvic acid content, 1 ml samples were diluted to 200 ml. The diluted juice (2 ml) was added to 1 ml of 0.0125% of 3–4-dinitro phenylhydrazine in 2 N HCl. After keeping for 10 min at 37°C, 5 ml of 0.6 NaOH was added and the absorbance of the solution measured with a Baush and Lomb colorimeter. The method was calibrated with sodium pyruvate as standard and the results expressed as mol of pyruvic acid per ml of juice. Estimation was done according to suggested methods[1,2]. The diallel analysis was carried out on the lines suggested earlier[3,4]. There were significant differences among the treatments but block differences were non-significant.

The estimates of $\hat{D}$, $\hat{H}_1$ and $\hat{H}_2$ were significant but $\hat{F}$, $\hat{h}^2$ and $\hat{E}$ were non-significant. The positive value of $\hat{F}$ indicated the more frequent occurrence of dominant genes in the parents than in recessive genes. The value of $\hat{D}$ was less than $\hat{H}_1$ which showed the presence of dominant genes more than additive ones. The mean degree of dominance $(\hat{H}_1/\hat{D})^{1/2}$ of 1.16 indicated dominance. The proportion of genes with positive and negative effects $(\hat{H}_2/4\hat{H}_1)$ was less than 0.25 which showed asymmetry at loci showing dominance. The proportion of dominant and recessive genes [$(4\hat{D}\hat{H})^{1/2} + \hat{F}/4\hat{D}\hat{H})^{1/2} - \hat{F})$ in the parents was 1.91 while the number of groups of genes showing dominance $(\hat{h}^2/\hat{H}_2)$ was 0.01 (table 2).

Table 2 *Estimates of variable components and some statistical parameters*

Statistics	Pungency (pyruvic acid)
$\hat{D}$	4.53** ±0.78
$\hat{F}$	3.27 ±1.83
$\hat{H}_1$	6.06* ±1.78
$\hat{H}_2$	4.86*' ±1.55
$\hat{h}^2$	0.06 ±1.04
$\hat{E}$	0.02 ±0.26
$(\hat{H}_1/\hat{D})^{1/2}$	1.16
$\hat{H}_2/4\hat{H}_1$	0.20
$\frac{(4\hat{D}\hat{H}_1)^{1/2}+\hat{F}}{(4\hat{D}\hat{H}_1)^{1/2}-\hat{F}}$	1.91
$\hat{h}^2/\hat{H}_2$	0.01
t^2	0.05

*P = 0.05 **P = 0.01

The above results indicate that the pungency (pyruvic acid) in onion was governed both by dominant and additive genes. The magnitude of the above components showed that the dominance components were higher than additive components. Significant improvement can therefore be achieved by heterosis breeding but the improvement of this character can also be undertaken by the conventional

Table 1 *Details of the parental lines*

Code and name of the parental inbred lines	P_1 'Pusa Red'	P_2 'Sel 106'	P_3 'Sel 131'	P_4 'Sel 102−1'	P_5 'Sel 134'	P_6 'Sel 126'	P_7 'Sel 96'	P_8 'Punjab selection'
Pungency (pyruvic acid) µmol/ml of juice	12.25	11.82	10.38	8.63	13.31	15.00	14.69	11.63
Colour of the bulbs	Red	White	white	Light-red	White	Yellow	White	Light-red

breeding methods. In onion, pungency is an important property, as Indians like the pungent onions. Pungency in onion is positively correlated with better storage and good drying ratio[5].

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RNA AS A SUBSTRATE FOR THE ASSAY OF HEAT STABLE NUCLEASE OF *STAPHYLOCOCCUS AUREUS*

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THERMONUCLEASE serves as a good index of enterotoxigenic staphylococci in foods. This assay has been used in screening foods for the possible presence of enterotoxins[1-3], especially in situations where the number of cells was known to decline during processing and storage[4,5]. The agar well diffusion technique is widely used for thermonuclease assay[6]. Due to difficulties in procuring calf thymus DNA, which is also expensive, in the present work yeast RNA has been used as a suitable replacement, as it is easy to prepare and more economical.

Ribonucleic acid was purified from commercial yeast preparation according to Woodwards[7]. Enterotoxigenic strains of *Staphylococcus aureus* (FRI, S-100 and S-361) were procured from Dr M.S. Bergdoll, FRI, USA. Crude enzyme was prepared from cell-free supernatant of 24-hr-old culture in brain heart infusion broth impregnated with 5% skimmed milk powder after adjusting the pH to 3.8. It was steamed for 20 min, cooled and centrifuged for 30 min at 6000 rpm. The clear supernatant was kept chilled and used within 2 hr.

Ten different food materials such as raw beef, canned corned buff, canned meat-in-gravy, seasoned multipurpose food, plain multipurpose food, vegetable protein convalescent food, cooked rice, salted-dried mackerel, cake and frozen mutton mince were used for the nuclease assay. Twenty grams of food sample were spiked with 0.2 to 2 ml crude enzyme of *S. aureus*. Five grams of skimmed milk powder and 30–70 ml distilled water were added and the entire mass was blended. The homogenate was steamed for 20 min, cooled and centrifuged. The clear supernatant was used for the assay.

Gel diffusion agar assay[5] medium was employed with slight modifications. To 100 ml of acetate, tris and phosphate buffers of varying molarity and pH were added the following: agar 1%, purified yeast RNA 0.05–0.1%, calcium chloride 0.003 moles % and toluidine blue 0.0003 moles %. For slide assay, 2.5 ml of the compounded medium was pipetted on to 25 × 75 cm glass slide and chilled to set the medium. Wells (2 mm diam) were punched and equilibrated to 50°C. The wells were filled with 5 μl food extract and the slides were transferred to air-tight humid plastic boxes. For petri plate assay, 5.5 ml medium was poured into 13 × 50 mm petri plates and 5 mm wells were punched and 30 μl sample fluid was added. Slides as well as petri plates were incubated at two different temperatures (37 and 50°C) for 2, 4 and up to 18 hr.

The results with acetate, tris and phosphate buffers with different molarities and pH are depicted in table 1. Of the three buffers, phosphate buffer at 0.2 and 0.1 M at pH 7.0 incubated at 50°C for 2 hr has given optimum results. For consistent results 0.1 M phosphate buffer at pH 5.5 is recommended. Two hour incubation was considered optimal since no additional benefit resulted by extending the incubation up to 4 hr and beyond. This reduces the conventional DNase assay period by 2 hr[6,8]. Yeast RNA at 0.05% concentration was found to be optimal. Positive assay was obtained with ten different foodstuffs inoculated with both *S. aureus* strains.

Calf thymus DNA is an expensive material for the assay of Staphylococcal nuclease. Since *S. aureus* secretes nuclease which hydrolyses nucleotide polymers into 3′-nucleotides by breaking the phosphodiester linkages[9] and the organism is also known to excrete RNases[10], the present results with yeast RNA were found convenient and cheap substitute for calf thymus DNA. Further, the difficulty in discerning colour change with DNA medium leading to inconsistent results[3] could be avoided. Petri plate assay was more convenient than microslides, as larger (30 μl) volume of the food extract could be