INHERITANCE OF SALT TOLERANCE IN PROGENIES OF TISSUE CULTURE SELECTED VARIANTS OF RICE

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TISSUE culture is now being widely employed to select for stress-tolerant variants in crop plants[1-4]. However, the variants generated by this technique are not always true-breeding, as they could have arisen from epigenetic or physiological causes[5]. Such variants may find no use in crop improvement and therefore it is important to identify true genetic mutants. This can be done by studying the stability of the altered trait through a few sexual generations[6,7]. In the present study 221 rice regenerants (R_0) were generated from control and stressed calli through somatic embryogenesis. The inheritance of salt tolerance in the first seed progeny (R_1) is reported in this paper.

Calli were induced from mature embryos of the salt susceptible varieties IR20 and IR50 in induction medium (MS (ref. 8) + 2 mg/l 2,4-D + 0.5 mg/l kinetin). The calli were then taken through six passages, each passage lasting 3–4 weeks, in salinized medium (MS + 2 mg/l 2,4-D + 0.5 mg/l kinetin + 10 g/l NaCl) and control medium (without NaCl). At the end of each passage some of the surviving calli in each variety were put in regeneration medium (MS + 2 mg/l kinetin + 0.5 mg/l NAA) for regeneration. The regenerants (R_0), after hardening, were grown in pots to maturity, and R_1 seeds collected from them individually. The R_1 progeny lines were raised in seed boxes (100 seeds per row) and when the seedlings were 15 days old, they were salinized with 0.5% NaCl solution. Resistant (Pokkali) and susceptible (IR20) checks were also sown in each seed box for comparison. When all the 'check' seedlings of IR20 were dead (after 5 days), the number of surviving R_1 seedlings was counted in each progeny row. The proportion of resistant to susceptible seedlings, based on seedling mortality, was calculated for each R_1 progeny row.

Of 221 lines, only two, viz. one of IR20, derived from callus stressed for three passages, and the other of IR50, derived from callus stressed for four passages, were found to be completely tolerant at the seedling stage. The appearance of these two variants can be explained by dominant mutations, as has been observed for brown spot tolerance in tissue culture selected rice variants[9]. In addition, 51 lines were found to be segregating for salt tolerance and susceptibility (table 1). In IR20, 20 lines derived from stressed calli and 9 from the control, and in IR50, 12 lines from stressed calli and 10 from the control segregated. All the other R_1 lines evaluated were found to be totally susceptible. Progenies with resistant variants seemed to be produced with higher frequency from the regenerants of stressed calli compared to those from control.

The pattern of segregation in each of the segregating R_1 lines of IR20 and IR50 showed that only 25% or less of the seedlings were resistant and this suggested a recessive bias for the mutation conferring salt tolerance.

None of the lines showed a strict Mendelian pattern of segregation. However, two lines each from

Table 1 *Number of segregating and non-segregating R_1 progenies in the salt tolerance screening trial*

Number of passages	IR20				IR50			
	Control		Stressed		Control		Stressed	
	S	NS	S	NS	S	NS	S	NS
1	1	20	3	14	1	16	1	13
2	1	16	5	6	2	9	2	7
3	1	8	3	5	1	5	1	6
4	3	6	3	4	2	5	3	3
5	2	5	3	3	2	4	2	3
6	1	5	3	3	2	3	3	1
	9	60	20	35	10	42	12	33

S, Segregating; NS, nonsegregating.

IR20 and IR50 broadly showed a 15:1 segregation (susceptible to tolerant), three lines from IR50 showed 13:3 segregation and another three lines from IR50 showed a 1:1 segregation at the seedling stage (based on survival). The resistant segregants have been transplanted to the field and are being grown to maturity to get the R_2 generation seeds.

Stable salt-tolerant variants have been generated through tissue culture in tobacco and *Citrus*[11]. In rice also, salt-tolerant variants have been generated through cellular level screening, using seawater[12] or NaCl[13,14] as stressing agents. The present study reaffirms the fact that salt-tolerant variants that may find use in crop improvement can be generated through callus level screening in rice. A systematic progeny evaluation would reveal the actual worth of these variants in developing salt-tolerant varieties.

16 December 1988; Revised 13 February 1989

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NEUROANATOMY AND CHOLINESTERASE ACTIVITY IN *ACANTHOTAENIA MULTITESTICULATA* (CESTODA)

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ACETYLCHOLINESTERAE (AChE, EC 3.1.1.7) has been shown in the nervous system in cestodes[1-4], nematodes[5,6] and trematodes[7]. However, comparatively less work has been done on this aspect in cestodes[8]. This communication reports work on AChE localization in the cestode *Acanthotaenia multitesticulata* (Proteocephalidae).

The parasites were collected from the intestine of the monitor lizard *Varanus bengalensis*. Immediately after removal from the host, the worms were washed twice in Tyrode's solution, pressed gently under a coverslip, and fixed in 10% formalin for 20 min. Following fixation the worms were washed in cold distilled water to remove all traces of the fixative. After washing, one group of worms was incubated in the medium of Holt and Withers[4], using 5-bromo-indoxyl acetate as substrate for 3–6 h at room temperature to detect non-specific esterases (NSE). Some worms were incubated in acetylthiocholine iodide (AThChI) to localize AChE following the direct colouring method of Karnovsky and Roots[5]. AChE activity was determined by its ability to hydrolyse the above substrates in the presence of pseudocholinesterase inhibitor tetraisopropyl pyrophosphoramide (10^{-5} μM)[9]. The possibility that other esterases besides cholinesterase could hydrolyse the substrates was eliminated in experiments using the specific cholinesterase inhibitor serine sulphate (10^{-5} μM).

The AChE activity in the parasites and in the medium was assayed colorimetrically. The enzyme assay and *in vitro* studies were carried out following the method of Gunn and Probert[3].

There are two small cerebral ganglia in the scolex, connected by a short cerebral commissure (figure 1). The two ganglia give rise to two median longitudinal