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THEORETICAL AND APPLIED GENETICS

Production of Triploid Plants from the Immature and Mature Endosperm Cultures of Rice

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Summary. Triploid plants were induced to regenerate from immature and mature endosperm cultures of three cultivars of rice. The plants were subsequently established in the soil. The significance of the induction of genetic variability in the germplasm of rice is discussed.

Key words: Tissue culture – Triploid rice plants – Genetic variability – Plant breeding

Introduction

Triploid and hexaploid plants are more vigorous than diploids (Morinaga and Fukushima 1935), and their foremost use is in the hybridization programs, especially for the augmentation of reservoirs of germplasm. Hybrid selection can then be made for the desirable agronomic traits (Khush 1975). Normally, triploids and hexaploids are produced by using conventional methods of plant breeding, which are time-consuming. However, by the in-vitro culture of endosperm they can be obtained in a matter of weeks.

Plant tissue cultures are a rich source of genetic diversity (Reinert and Bajaj 1977), and their importance in the artificial induction of genetic variables in rice improvement programs is fairly well proven (Bajaj 1980). In-vitro induced genetic diversity in tissue cultures augments the naturally occuring pools of germplasm to increase the production capacity of crops (Wittwer 1974). In the present investigation, the results on the regeneration of triploid plants from cultures of excised endosperm of three cultivars of rice are summarized.

Material and Methods

The surface sterilized seeds of three cultivars ('Basmati 370', 'IR 36', 'HM 95') of rice (*Oryza sativa* L.) were soaked in sterile distilled water for 24 hours. The part of the seed containing the embryo was excised and discarded. The remaining portion of the endosperm was cut into three pieces and cultured on Murashige and Skoog's medium (Murashige and Skoog 1962) containing various combinations and concentrations of kinetin, IAA, 2,4-D, and yeast extract. Likewise, the immature endosperm was dissected out from young developing spiklets (4-8 days after pollination), and 2 or 3 explants were cultured per tube. All manipulations were conducted under sterile conditions in a Laminar Flow Chamber (Klenzaids, Bombay), and the cultures, incubated at 23°-26°C in diffused light, were observed periodically.

When the plants had developed a sufficient number of roots (Fig. 4), they were taken out of the tubes, and the agar was gently removed under running tap water. The plants were then transferred to pots (Fig. 5), containing autoclaved soil.

For cytological studies, the root tips and callus cells were pretreated with a saturated solution of α-bromonaphthalene for 3-4 hours, fixed in acetic alcohol and subsequently stained with Feulgen and Carmine.

Results and Discussion

There was a striking difference in the growth response of immature and mature endosperm of various cultivars of rice cultured on different media. The immature endosperm underwent two modes of differentiation, i.e. direct regeneration of plants without the intervening callus phase, and indirect through the differentiation of callus. The mature endosperm, however, first proliferated to form callus, and later the plants differentiated in 4-6 weeks. (Figs 1-6).

On Murashige and Skoog's medium + 2,4-D (2mg/l), the segments of mature endosperm started to proliferate in 3-7 days (Fig. 1), and a mass of callus was formed in 3 weeks. Occasionally rhizogenesis was observed in some cultures but there was no shoot formation. The callus was mostly nodular (Fig. 2), soft, and creamy. It has been subcultured, and maintained on MS + 2,4-D (1mg/l). The callus, on transferring to MS_3 medium (MS + IAA 4mg/l + kinetin 2mg/l), started to differentiate within 2 weeks,

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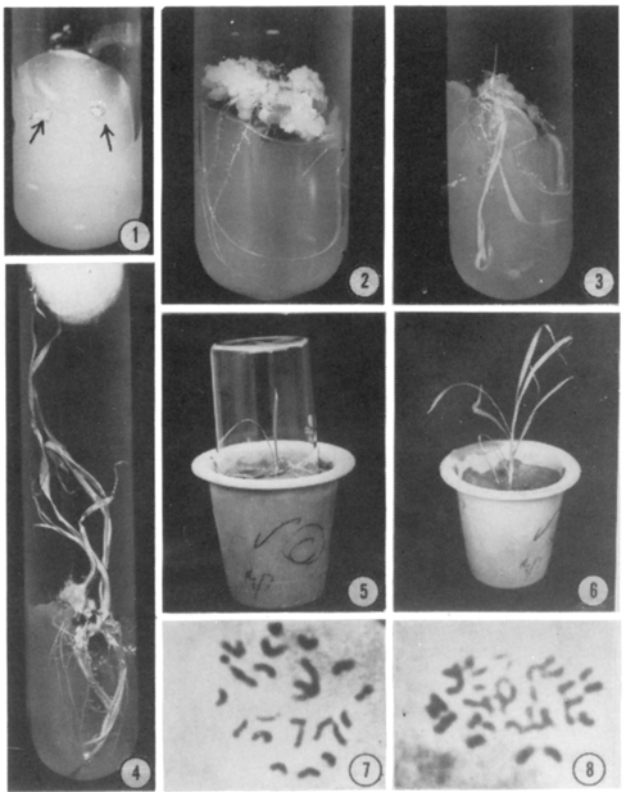

Figs. 1-8. In vitro regeneration of plants from rice endosperm. 1 Endosperm explants, 6 days after inoculation on MS + 2,4-D, showing the initiation of callus (marked with arrows). 2 Profuse proliferation and rooting in callus 2 weeks after subculturing on MS_3 medium. 3, 4 Differentiation of plantlets from endosperm-derived callus of cv. 'Basmati-370', 3 and 6 weeks after subculturing on MS_3 medium. 5, 6 Plantlets, 1 and 5 weeks after transferring to soil. 7 Root tip squash from a normal embryo-derived diploid (2n = 24) plant. 8 Endosperm callus cell with triploid number (3n = 36) of chromosomes

and complete plants were obtained within 4-6 weeks (Figs. 3, 4). The cultures showed various modes of differentiation i.e. only roots, shoots, or both.

The proliferation of immature endosperm and the occasional formation of shoots occured in a medium fortified with yeast extract (Nakano et al. 1975). However, a combination of IAA and kinetin induced and enhanced shoot formation. The triploid plants (Figs. 5, 6, 8) produced from the endosperm showed broader leaves, a faster rate of growth, and more of tillering than the embryo-derived plants (diploids Fig. 7).

In cultures, the endosperm from various cultivars showed differential genotypic responses. As judged by their growth and the time taken for the formation of plants, the response was best in the case of cv. 'B-370', followed by 'HM 95' and 'IR 36'.

In addition to triploids a large variation in the number of chromosomes in the mature endosperm-derived (via callus) plants was observed. Among them, aneuploids and polyploids were common.

This study demonstrates the possibility of augmentation of the depleting gene pool in rice through artificial incorporation of genetic diversity by in-vitro means.

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