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Brassinosteroids and benzylaminopurine increase yield in IR50 indica rice

Brassinosteroids (BR) are naturally occurring steroidal plant growth regulators. Since the isolation of brassinolide from rape pollen in 1979, more than 40 natural analogs have been reported to be widespread in the plant kingdom[1]. In a variety of bioassays, BR are active at picomolar and nanomolar concentrations, as against micromolar concentrations of other plant growth regulators (PGRs). Physiological and molecular genetic studies support the view that BR should be considered as a new class of plant hormones[2–5]. BR promote elongation, division and differentiation of cells, and ethylene biosynthesis. They enhance auxin-mediated bending of rice leaf lamina[6] and growth of grass leaf sheath pulvinus[7]. External application of BR is reported to increase yield in vegetable and fruit crops and enhance resistance against salt, herbicides and fungal pathogens[8,9].

We investigated the effects of brassinosteroids and several other plant hormones and other PGRs on the vegetative and reproductive growth of IR50 rice. This report focusses on the promising results of yield increase obtained with BR and BAP.

Seeds of *Oryza sativa* var. indica cv IR50 were obtained from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Rice plants were grown in pots in the nursery and in fields (2 × 2 m experimental plots) in the college farm. Desired concentrations of PGRs were prepared from stock as aqueous solutions containing a few drops of Tween 20 or Triton-X. The PGRs were applied as soaking spray treatments at various stages of development like vegetative, panicle initiation and anthesis[10]. The control plants were sprayed with water containing a few drops of the above detergents. Brassinolide was obtained from Zen-Noh, National Federation of Agricultural Cooperative Association, Tokyo, Japan. A synthetic isomer that gave similar results was supplied by W. J. Meudt of USDA, Beltsville, Maryland. All other PGRs used in this investigation were obtained from the Sigma Chemical Company, USA.

Periodic measurements were made on plants growing in the field and pot cultures. Fresh and dry weight of mature spikelets were determined for 1000 grains[11]. The data were analysed by the ranking of the treatment means. ANOVA Test and Duncan Multiple Range analyses were carried out to determine whether the results obtained were statistically significant. Free-hand

Table 1. Effects of PGRs on tillering, spikelet number, grain-filling and yield in IR50 rice†

Treatment molar (M)	Number of fertile tillers	%	Number of spikelets	%	Number of filled spikelets	%	Fresh weight of 100 grains (g)	%	Dry weight of 100 grains (g)	%
Control	8.0^{d}	–	74.30^{d}	–	63.70^{f}	–	2.316^{e}	–	2.158^{f}	–
Brassinosteroid (BR)10	13.5^{a}	68.7	110.40^{a}	48.5	104.6^{a}	64.2	2.853^{b}	23.2	2.728^{b}	26.4
Benzylaminopurine (BAP)10^{-5}	9.8^{c}	22.5	85.80^{c}	15.4	81.10^{e}	27.3	2.709^{c}	16.9	2.610^{c}	20.9
Gibberellic acid (GA_3)10^{-6}	6.6^{e}	−17.5	121.30^{a}	63.2	93.30^{c}	46.46	2.557^{d}	10.4	2.462^{e}	14.0
Kinetin (KIN) 10^{-5}	10.0^{c}	25.0	94.20^{b}	26.7	87.20^{d}	36.89	2.730^{c}	17.8	2.670^{b}	23.7
BR 10^{-7} + KIN 10^{-5}	11.9^{b}	48.7	97.40^{b}	31	91.90^{c}	44.27	2.842^{b}	23.0	2.738^{b}	26.8
BR 10^{-7} + BAP 10^{-5}	12.8^{a}	60.0	107.90^{a}	45.2	102.20^{a}	60.04	3.119^{a}	34.7	2.999^{a}	38.9
BR 10^{-7} + GA_3 10^{-6}	6.8^{e}	−15.0	121.40^{a}	63.4	89.0^{d}	39.7	2.717^{c}	17.3	2.538^{d}	17.6

%Promotion over control.
†Identical letters following the values indicate no significant difference according to Duncan Multiple Range Test ($P < 0.05$).

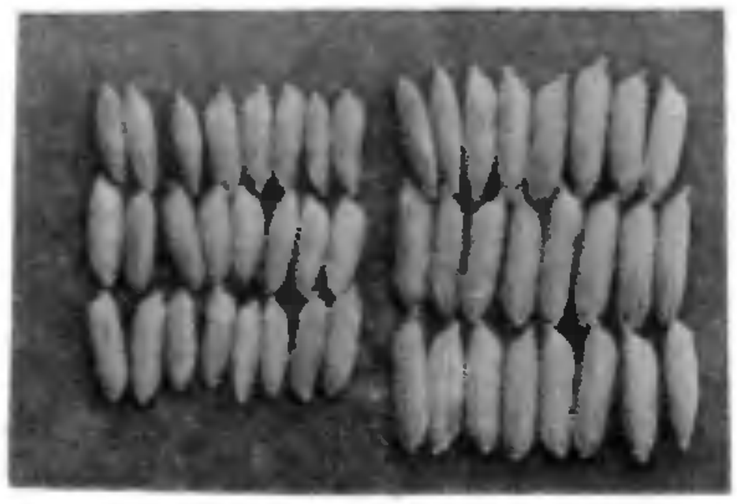

Figure 1. Control rice grains (left) and grains from brassinosteroid and benzylaminopurine sprayed plants.

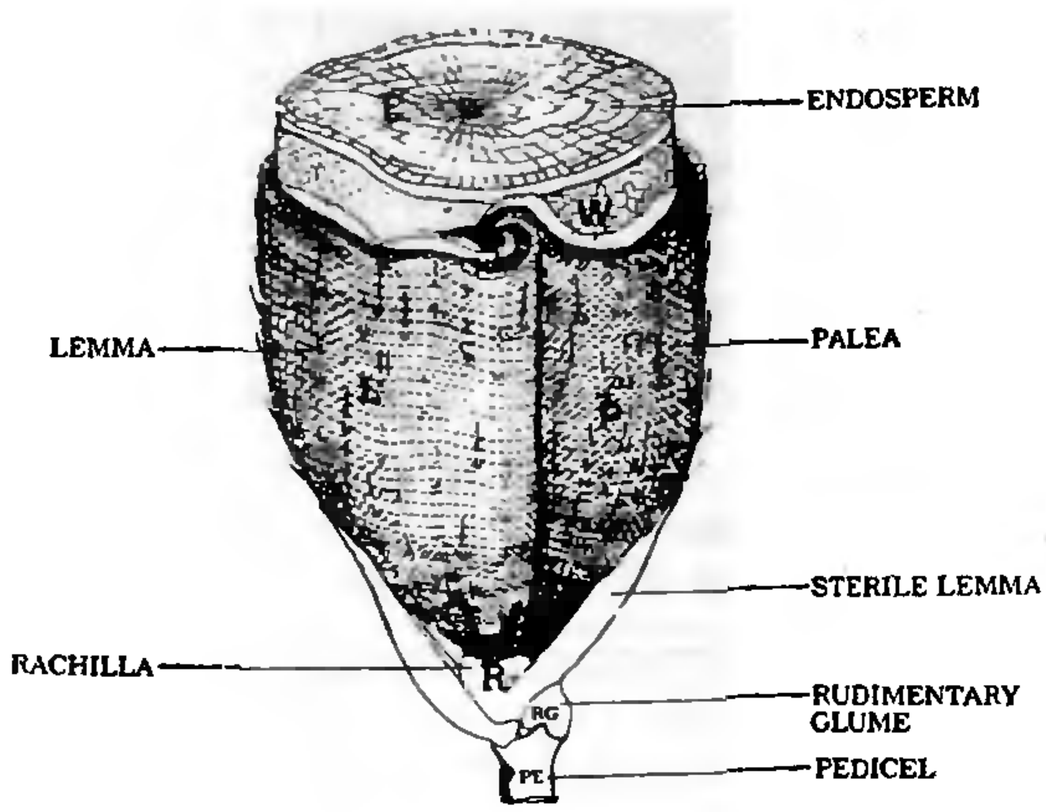

Figure 2. Perspective diagram of a rice grain. The caryopsis is tightly enclosed by a lemma and palea which impose size restriction on the caryopsis. Other associated structures are the rachilla, sterile lemmas, rudimentary glume and pedicel. An abscission layer is located at the base of the sterile lemmas.

and microtome sections were stained with bright-field and fluorescent dyes to determine cell number and size and to localize major storage reserves, namely, starch, proteins and lipids[12].

The results obtained with experimental field plots are summarized in Table 1, and are confined to those parameters that directly contribute to yield enhancement. Information on the effects of the PGRs on vegetative growth is not reported in this communication. Control plants possess an average of 8 fertile tillers per hill. The average number of fertile tillers is increased to 13.5 by BR. Both kinetin (KIN) and benzylaminopurine (BAP) inhibit the effect of BR although independently they tend to enhance the tiller number. Gibberellic acid (GA_3) significantly decreases the number of fertile tillers from 8 to 6. The addition of BR does not overcome the suppressive effect of GA_3. Both BR and GA_3 promote the number of spikelets differentiated per hill to 46% and 63%, respectively. However, the number of filled spikelets is more in BR-treated plants than in GA_3-treated ones (105 vs. 93). The cytokinins KIN and BAP do not strongly influence the differentiation of spikelets.

The fresh and dry weights of control grains are 23.16 mg and 21.58 mg/grain, respectively. Fresh grain weight increases maximally to 31.19 mg/grain when treated with BR and BAP. Against this increase of 34.6%, the addition of GA_3 and KIN to BR promotes fresh weight only by 17% and 23%, respectively. BR promotes dry grain weight by 26.4%. A significant increase of 39% dry grain weight is induced by the combined action of BR and BAP.

The length, width and thickness of the control grains are 8.5 mm, 2.7 mm and 1.8 mm, respectively. BR + BAP treatment enhances grain size by affecting all three dimensions (10.6 mm, 3.1 mm and 2.2 mm). The grains thus appear larger and bolder (Figure 1). In BR and BAP-treated plants, the palea and lemma are large even at the time of anthesis. Treatment also increases the number of aleurone cells from 202 in control to 280 per cross-sectional area. The aleurone cells occur mostly in a single layer surrounding the endosperm cells. The endosperm cell number (as seen in cross-sectional area) is also increased from 265 in control to 400 in treated caryopsis. Careful microscopic analysis reveals that a control grain has an estimated total number of 75,400 endosperm and aleurone cells. A BR + BAP treated grain is estimated to possess a total of 160,000 cells. The size of an endosperm cell also increases by 40% in BR + BAP treatment.

Histochemical studies of control and BR + BAP treated grains reveal that increase in size of the grain does not alter the patterns of distribution of major storage materials. In both grains aleurone grains and lipid occur mostly in the aleurone cells and in the embryo. Three different kinds of protein bodies are detected both in control and treated grains, and they occur mostly in the subaleurone layers. The bulk of the grains is made up of starch[12].

Unlike other major cereal grains the edible part of rice, the caryopsis, is tightly covered by several sterile glumes (Figure 2). These structures, especially the palea and lemma, impose a restriction on the size of the caryopsis[13]. Grain yield in rice is a function of the number of panicles per hill and the number and weight of filled spikelets. The weight of a spikelet (grain) is the most recalcitrant of these yield components. A larger and a heavier caryopsis can be obtained only when the space enclosed by the palea and lemma is larger, thus permitting the caryopsis to enlarge and fill this space (Figure 2). However, the palea and lemma are fully developed even at the time of anthesis. These structures are generally insensitive to external application of PGRs[13]. Any attempt to alter the size of the palea and lemma should be carried out during early stages of panicle initiation and not at anthesis. It is significant that BR+BAP applied during the panicle initiation phase does indeed affect the size of these hull components. The lemma elongates by 25%, from 8.5 mm in control grains to 10.6 mm in treated plants.

Development of the caryopsis is a post-anthesis phenomenon and can be influenced only if BR and BAP are applied immediately after anthesis. Previous pot culture experiments have shown that auxin and triacontanol-based products promote yield in rice. Of all the PGRs that we investigated, BR appears to be the most promising especially at 10^{-7} M with 10^{-5} M of BAP.

The pot culture and small field plot experiments should be extended to field trials to assess the usefulness of the protocols for farm-level yield enhancement in rice. Also, since the enlargement of the caryopsis of most other cereal grains is not restricted by sterile glumes as in the case of rice, BR and BAP may be expected to more readily promote size/weight in these other cereal grains.

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In vitro multiplication of *Centella asiatica*, a medicinal herb from leaf explants

Plant-based remedies have always been an integral part of traditional medicine throughout the world. The increasing demand for herbal medicines in recent years due to their fewer side effects in comparison to synthetic drugs and antibiotics, has highlighted the need for conservation and propagation of medicinal plants. Tissue culture provides efficient techniques for rapid and large-scale propagation of medicinal plants and their *in vitro* conservation of germplasms.

Centella asiatica L. (Apiaceae) is an important medicinal plant used in several ayurvedic preparations and is reported to possess antileprotic, antifilarial, antifeedant, adaptogenic, antiviral and antibacterial properties[1,2]. It is also reported to possess insecticidal properties[3]. The plant contains several triterpene saponins, e.g. asiaticoside and sapogenins, the bitter principle vellarin, the glycosides, centelloside, the alkaloid hydrocotylin amongst other components[4,5]. The requirement of *C. asiatica* is now met from the natural populations, leading to their gradual depletion. Tissue culture techniques can play an important role in the rapid multiplication of elite clones and germplasm conservation of *C. asiatica*. Furthermore, there is a wide scope for application of biotechnology for improvement of this important medicinal plant for which standardization of an efficient direct *in vitro* multiplication protocol is a crucial prerequisite. The present paper, to our knowledge, reports for the first time, a simple and rapid method for the *in vitro* multiplication of *C. asiatica* from leaf explants and soil establishment of plants.

Leaf explants, collected from 5–6 months old glasshouse grown plants of *C. asiatica* were initially washed with a detergent solution (1% teepol) for 2–3 min, followed by thorough washing under running tap water for 30 min. The explants were then surface sterilized with an aqueous solution of 0.1% (w/v) $HgCl_2$ (HiMedia, India) for 2–3 min, followed by a final 5–6 rinses with sterile double distilled water. The sterilized leaf explants, dissected into two halves with or without petioles, were cultured on modifications of Murashige and Skoog's (MS) medium[6] containing different concentrations and combinations of cytokinins (0.5 to 3.0 mg/l) – 6-furfuryl benzylaminopurine (BAP) and kinetin (Kn) and auxins (0.01 to 0.1 mg/l)–indole butyric acid (IBA) and naphthaleneacetic acid (NAA). All the cultures were maintained at 25 ± 2°C, under continuous light of 3000 lux and with 55–60% relative humidity.

Leaf segments devoid of petioles were more responsive than those with petioles. BAP (2 mg/l) along with IBA (0.1 mg/l) produced maximum sprouting where 80% of the leaf segments devoid of petioles showed initiation along the margins and cut ends (Figure 1 *a*) within two weeks, and only 30% of leaves with petioles responded, showing initiation near the distal cut ends of petioles after 4 weeks of culture initiation (Figure 1 *b*). On the other hand, combinations with Kn failed to show any response and resulted in yellowing of the explants. The superiority of BAP over Kn in multiple shoot induction was also reported earlier in a number of medicinal plants[7–10]. Although the initial sprouting required the presence of BAP at 2 mg/l