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## Plant regeneration in barley through microtillering and multiple shoot differentiation

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Shoot multiplication was obtained through microtillering as well as multiple shoot formation in barley. Microtillering occurred from cultured immature embryos on MS and B5 media supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin, whereas from the cultured mature embryos shoot multiplication was through formation of *de novo* multiple shoot buds via an intervening callus phase. In the subsequent subcultures the number of shoots increased. Rooting of regenerated shoots was achieved on basal MS or B5 medium. Rooting was better on B5 medium in comparison to MS. The regenerated plants were transferred to field conditions where they flowered and set seeds.

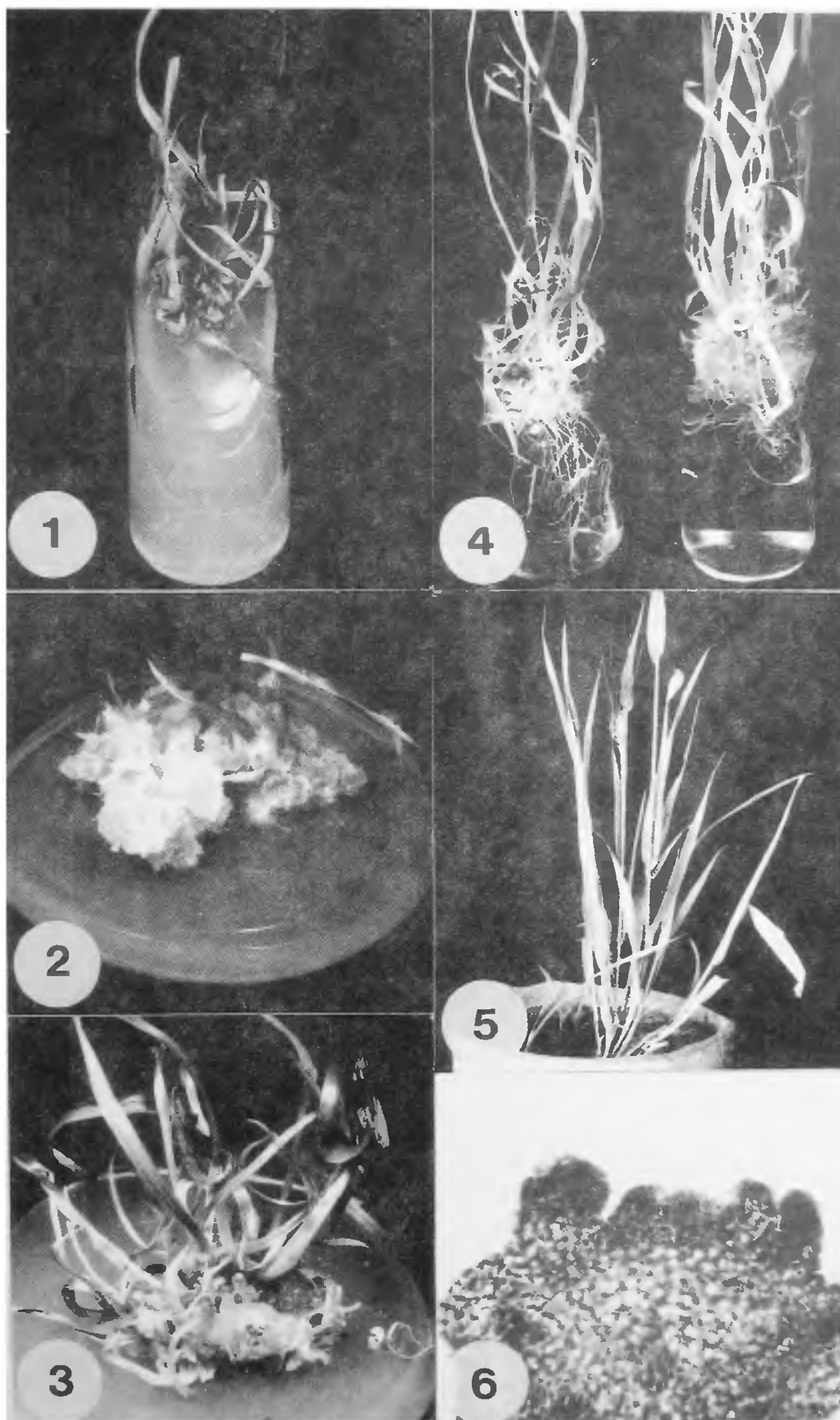
PLANT regeneration in cereal tissue cultures can follow two different pathways, i.e. organogenesis or embryogenesis<sup>1-6</sup>. Organogenesis or shoot morphogenesis involves the development of *de novo* organization of shoot meristems in callus cultures. While somatic embryogenesis is the most common pathway of regeneration in most cereals, barley has been reported to be recalcitrant with regard to somatic embryogenesis or stable regeneration of plants through organogenesis<sup>7,8</sup>. Microtillering and multiple shoot formation as a method of shoot multiplication has been described

in sorghum<sup>9-11</sup>, wheat<sup>12,13</sup> and finger millet<sup>14,15</sup>. This paper describes microtillering from immature embryos of barley and formation of multiple shoots in callus cultures of mature embryos.

Seeds of barley (*Hordeum vulgare* L. genotype BL-2) were obtained from the Agriculture Research Station, Durgapura, Jaipur. Seeds were field grown for immature embryo culture experiments. Young spikes 15 to 18 days after anthesis were harvested. Both the young spikes and the seeds were surface sterilized in 0.1% HgCl<sub>2</sub> (w/v) solution for 3-5 minutes and rinsed 3-4 times in sterile distilled water. While immature embryos ranging in size from 0.5 mm to 1 mm were gently excised immediately following several rinses with sterile distilled water, mature embryos were excised only after soaking the seeds in water for 48 h. The immature and mature embryos were cultured on MS medium<sup>16</sup> containing 3% sucrose and 0.8% agar (Qualigens) and B5 medium<sup>17</sup> with 2% sucrose and 0.8% agar. Both MS and B5 media were supplemented with 2.5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 mg/l kinetin. The cultures were incubated at 26 ± 2°C in the dark. Callus cultures of mature embryos with shoot buds were transferred to medium containing lower levels of 2,4-D in combination with kinetin for their multiplication. Elongated shoots were cultured on basal MS and B5 media. Thirty green plants were transferred to pots in the field directly without any previous hardening and all of them survived and grew to maturity.

After 10 days of culture, immature embryos formed 3-8 shoots on media containing 2.5 mg/l 2,4-D and 0.5 mg/l kinetin. Within 30-35 days the cultured immature embryos formed more than 8 shoots through





Figures 1–6. 1, Microtillering from cultured immature embryo at 2.5 mg/l 2,4-D and 0.5 mg/l kinetin on B5 medium after 20 days, 2, Induction of callus along with few shoots from mature embryo at 2.5 mg/l 2,4-D and 0.5 mg/l kinetin on MS medium in primary culture after 15 days, 3, Multiple shoot formation from callus culture of mature embryo at 0.5 mg/l 2,4-D and 0.2 mg/l kinetin in third subculture on MS medium, 4, Root formation in B5 medium devoid of growth regulator, 5, *In vitro* plant growing in the field condition, 6, Mature-embryo-callus showing shoot buds in a section



**Table 1.** *In vitro* response of mature embryos of barley on MS and B5 media with 2,4-D + kinetin

Medium	Embryos plated on medium containing 2,4-D (2.5 mg/l) + kinetin (0.5 mg/l)	Embryos producing callus	Calli producing green shoots on medium with lower 2,4-D (0.5 mg/l) + kinetin (0.2 mg/l)	Total no. of green shoots produced in three subcultures on 2,4-D (0.5 mg/l) + kinetin (0.2 mg/l)
MS	22	20	20	456
B5	20	20	20	480

microtillering. Proliferation of shoots occurred without any intervening callus through proliferation of axillary buds by a process called microtillering. There was no marked difference between MS and B5 media with regard to shoot formation. In both the media, after 35 days, 8–10 shoots were formed. All these shoots were green and no albino shoots were observed (Figure 1). The regenerated shoots were rooted both on basal MS and B5 media. Rooting was better on basal B5 medium compared to MS. The rooted shoots were transferred to pots in the field conditions, directly without any hardening treatment in the months of October and November, after which they matured and set seeds.

The cultured mature embryos induced 3–5 shoots in 7–10 days with intervening callus on both MS and B5 media containing 2.5 mg/l 2,4-D and 0.5 mg/l kinetin (Figures 2 and 6). Callus was creamish-yellow, friable, and non-embryogenic in nature. There was no marked difference in the morphology of shoot-forming callus and non-shoot forming callus. Callus and shoots were separated and the shoots were kept on both basal MS and B5 medium for rooting. The separated calli were grown on a medium containing low levels of 2,4-D (0.5 mg/l) and kinetin (0.2 mg/l) under 16 h photoperiod (1400 Lux). Under these conditions the callus continued to produce multiple shoots up to 3–5 subcultures after which callus growth and differentiation ceased (Figure 3). The number of shoots (more than 30) produced was maximum in the third and the fourth subcultures. However, the callus under any condition could not be maintained or induced for further growth. No albino-shoot was observed in any of the cultures. The difference in percentage response and shoot morphogenesis between MS and B5 media was marginal (Table 1). However, rooting was better on basal B5 (Figure 4) medium in comparison to MS medium. The plantlets were transferred to pots under field conditions where they survived and set seeds (Figure 5). A 15-day pretreatment of regenerated shoots prior to field transfer with 2 mg/l IBA as reported by Bhattacharya<sup>18</sup> was not necessary in our work.

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## Short-term storage of coconut zygotic embryos in sterile water

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**Coconut zygotic embryos (cultivar West Coast Tall) can be stored for two months in sterile water, Eeuwens Y3 media without charcoal or Y3 media without sucrose. When the embryos were transferred to the retrieval media, respectively 80.0, 66.7 and 66.7% germination were observed. This is the first report of the use of sterile water as the storage medium for coconut embryos.**

EXCHANGE of coconut germplasm between different countries as well as from distant places within the same country is often beset with problems arising from the large nut size, short duration of dormancy and phytosanitary regulations. These difficulties can be largely overcome if the embryos alone can be scooped out, stored in aseptic conditions and germinated later *in*

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