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Inorganic nitrogen stress, an additional pre-treatment to improve anther culture efficiency of *indica* rice variety Bg 358

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Anther culture is a powerful tool to produce di-haploid plants. Stress pre-treatments applied to anthers play a crucial role in switching the development of a microspore within the anther from normal gametogenesis towards sporophytic haploid plant development during anther cutlure. The present study attempted to test the effectiveness of combining the regular cold stress appliedon rice anthers (10 ^{0}C for 10 d) with the additional stress of nitrogen starvation, on the anther culture response of the *indica* rice variety Bg 358.

The cold-stressed anthers were further subjected to nitrogen starvation before culturing on normal N6 medium, by first plating anthers for 3 days on N6 callus induction medium with reduced levels of inorganic nitrogen. Four treatments of nitrogen stress were applied by complete or partial withdrawal of either or both $(NH_4)_2SO_4$ and KNO_3.

The highest callus induction frequency (3.73%) was recorded from anthers stressed with total withdrawal of $(NH_4)_2SO_4$, followed by anthers on the medium without KNO_3 (2.31%). Medium with half strength $(NH_4)_2SO_4$ & KNO_3 produced a lower callus induction frequency (1.97%) which was marginally better than the control treatment having normal levels of nitrogen (1.82%). Anthers on medium without inorganic nitrogenfailed to produce calli at all.
A positive effect on shoot regeneration was seen in callus developed on nitrogen starved media. The best regeneration response was observed in anther-derived calli grown on medium without $(NH_4)_2SO_4$, in terms of the shoot induction frequency (60.87%) and green shoot regenerability (56.52%). The two other conditions of nitrogen starvation (i.e. without KNO_3 and with half strength $(NH_4)_2SO_4$& KNO_3) also resulted in the improvement of green shoot regeneration (> 33%) compared to the control (18%), in spite of a higher total shoot regeneration frequency (45.45%) in the control of which a greater proportion was albinos.

Thus, it may be concluded that stressing anthers on $(NH_4)_2SO_4$ or KNO_3free medium for an initial 3 d period is beneficial for callus induction, shoot regeneration and green plant regeneration compared to the control, although the NO_3^- stress is less effective than the NH_4^+ ion starvation. However complete removal of both NH_4^+ and NO_3^- ions was detrimental for callus induction of rice microspores. A major problem in indica rice anther culture is the frequent regeneration of albino plants. Therefore, subjecting anthers to inorganic nitrogen stress will be a useful pre-treatment for the recovery of green plants.

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