In Vitro Manipulation of 2,4-D for Callus Induction and its Subsequent Regeneration in Selected Wheat and Rice Cultivars

Hamid Rashid & Azra Quraishi *

ABSTRACT

In tissue culture studies 2,4-Dicholorophenoxy acetic acid is extensively utilized both for callus induction and its regeneration, the optimum doses however, vary from cultivar to cultivar and from species to species. In rice, the cultivars studied were IR-6 and KS-282. It has been noted that embryogenic callus induction was possible when 2,4-Dicholorophenoxy acetic acid was utilized at low levels (0.5 mg/l and 1 mg/l) in combination with Benzylamino purine (BAP). Wheat cultivars Pak-81, Lyp-73 and Pavon-76 require relatively higher doses of 2,4-Dicholorophenoxy acetic acid (2-4mg/l) singly for embryogenic calli.

INTRODUCTION

An important factor controlling the morphogenetic responses of growth regulators is the availability and non-availability of auxins in the cells and tissues. In fact, the most relevant breakthrough in the development of plant tissue culture techniques was the discovery of morphogenetic activities of auxins, particularly those of the native and synthetic auxins and cytokinins. Auxins are thought to control the following procedures (a) apical dominance (b) cell elongation in roots and shoots (c) induction of adventitious root formation (d) enhancement of respiration rate (e) induction of disorganized growth at higher concentration (f) inhibition of embryo formation in cell suspension culture (g) mitotic irregularities in long term cultures (h) delay in senescence activities of the cells. The auxins most commonly used in a tissue culture system are Indole acetic acid (IAA), Naphthalene acetic acid (NAA) and

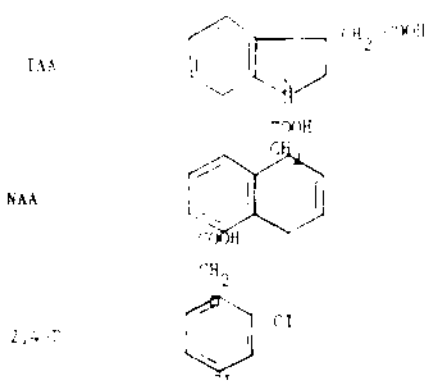

Fig-1 The major auxins used in plant tissue culture: IAA (native auxin), NAA, and 2,4-D (synthetic auxins).

2,4-Dicholorophenoxy acetic acid [Fig-1] (Jacobsen 1983).

In tissue culture studies auxins particularly 2,4-D is widely used for callus induction which has great significance for crop improvement. Within a callus it is always possible to distinguish several distinct cell popula

* National Agricultural Research Centre, Islamabad.

tions attributable to genetic and/or epigenetic changes. A general view of callus proliferation was presented by Yeoman (1971). Hill (1967) pointed out the need for wounding explant tissue, after which cell divisions proceed. Not all cells in the explant divide and mainly those in the outer layers of tissues contribute to the developing callus. Cell division activities is de-pen-dent on growth regulator concentration, as well as the source of explant material.

The objective of this study was to explore the effect of 2,4-D in selected rice and wheat cultivars for callus initiation, maintenance and regeneration in long term cultures for the retrieval of useful mutants.

MATERIALS & METHODS

Rice varieties selected for this study were IR-6 and KS-282 and wheat varieties utilized were Lyp-73; Pak-81 and Pavon-76. In either case the seeds were washed thoroughly with running tap water. Rice seeds were dehulled and sterilized with chlorox bleach for 15 minutes, followed by three subsequent rinsings with autoclaved distilled water under aseptic conditions. The wheat seeds on the other hand were dipped intact in a commercial detergent zip. Excess detergent was removed by thorough washing in tap water followed by several rinses in distilled water. The material was then sterilized first with a quick washing with 70% ethanol followed by a twenty minute sterilization with chlorox bleach containing 5.25% sodium hypochlorite. Excessive bleach was washed with sterile distilled water under aseptic conditions at least five times successively for fifteen minutes each.

The rice cultures were inoculated on modified MS media supplemented with sucrose, yeast extract, casein hydrolysate and varying concentrations of 2,4-D, NAA and kinetin with 0.8% agar. The wheat cultures were inoculated on modified MS medium supplemented with 3.0% sucrose, 0.8% agar and varying concentrations of growth regulators. The pH of the medium was adjusted to 5.8 prior to autoclaving. The media was autoclaved at 15 psi for 20 minutes.

The cultures were incubated both in dark and light and the photoperiod was adjusted for 16 hours/day with a light intensity corresponding to 1500 lux.

RESULTS & DISCUSSION

Rice: There are reports that 2,4-D concentrations in the medium are necessary for the induction of callus from different explant sources in rice cultivars. In our study, calli started initiating after one week of culture in IR-6 on media with 2,4-D concentrations at the rate of 0.5, 1.0 and 2.0 mg/l [Fig-2]. Slightly high concentrations of 2,4-D (2 mg/l) increased callus formation which was short lived. Media with 2,4-D (1-2 mg/l) and BAP 1-2 mg/l showed maximum calli with shoot primordia. Percentage of callus formation in all combinations ranged between 89.1% (2.0 mg/l 2,4-D and 1.0 mg/l BAP and 57.8% (0.5 mg/l 2,4-D). Apparently BAP had no sig-

Fig-2. Callus formation in IR-6

Fig-3. Multiple shoot formation in mature embryogenic callus in KS-282

nificant effect on callus induction. It improved callus size and determined its type, whether non-regenerative or otherwise.

Calli from all these combinations were transferred to regeneration medium which was MS supplemented with sucrose 10 g/l, casein hydrolysate and yeast extract 3g/l with kinetin 5 mg/l, BAP 2mg/l and NAA 0.1 mg/l. Maximum regeneration was observed in combination with 2,4-D 0.5 mg/l and BAP 1mg/l. Visible shoots appeared within one week of transfer [Fig-3].

It can be extracted from our study that 2,4-D and a cytokinin, BAP, were essential for callus induction in seed derived calli from IR-6. Brar et al., (1986) also reported that the presence of a cytokinin either BAP or kinetin was essential in promoting plant regeneration from cultured cells.

In KS-282 initiation of callus was observed after 3-5 days on modified MS (Murashige & Skoog 1962). Medium containing casein hydrolysate, yeast extract at the rate of 2g/l with varying concentrations of 2,4-D, 0.5, 1.0 an 2.0 mg/l alone or in combination with BAP at the rate of 1 and 2.0 mg/l. Callus induction frequency increased with increased levels of 2,4-D. Benzyl aminopyrine had no major effect on callus induction but it favoured significant increase in callus size and the ratio of embryogenic calli to non-embryogenic calli.

Wheat: Using 3 cultivars of wheat Pak-81, Lyp-73 and Pavon-76, the effect of 2,4-D was tested for callus induction both in dark and light. Callus initiation was observed after one week of culture in all the varieties tested irrespective of the light or dark period. [Fig 4].

Fig-4: Embryogenic callus formation in Lyp-73.

Lower concentration of 2,4-D (0.5 mg/l) failed to induce callus. Callus formation at higher concentrations of 2,4-D was variety specific. The amount and size of callus varied from variety to variety. In Pavon-76, callus was observed on media containing 2,4-D at the rate of 2 mg/l in dark. High concentrations of 2,4-D i.e. 1mg/l had an adverse effect. Callus induction frequency in light was less in all the combinations. In Lyp-73, 3mg/l 2,4-D produced good callus induction in light with less difference in the callus induction frequency at 3 and 1mg/l 2,4-D. In darkness there was a significant decrease in callus induction in all combinations. In Pak-81, callus induction frequency was high at 1mg/l 2,4-D in dark. At the rate of 2mg/l and 3mg/l 2,4-D, very slight difference in callus induction frequency was observed in light and dark. The frequency of good callus induction was noted to be highest in Pavon-76, followed by Lyp-73 and Pak-81. The frequency of embryogenic callus was less during the first passage, however, during subsequent passages, its frequency increased substantially. These results were in accordance with the work reported by Mackinnon et al. (1987).

The performance of callus after subculturing was studied using different media combinations. When the calli of Pavon-76 were subcultured on media with 2mg/l 2,4-D good proliferation of tissues was noted. When the concentration of 2,4-D was lowered from 3mg/l to 0.5mg/l proliferation was profuse. At elevated levels of 2,4-D i.e. 1mg/l, proliferation decreased and embryoid like structures were clearly visible thereby indicating that such levels could initiate differentiation. For Lyp-73 the optimum concentration of 2,4-D for good callus formation was 3mg/l. Pak-81 could be very well maintained on media supplemented with 1mg/l 2,4-D. The calli appeared regenerative with a good proliferation. Nabors et al. (1985) reported non-embryogenic callus not to be regenerable. In this study also when the non-embryogenic callus was transferred to the regeneration medium only slight differentiation was observed. The presence of IAA with BAP 5mg/l in the regeneration medium directed multiple shoot formation (Fig-5).

Fig 5: Regeneration from embryogenic callus of Lyp 73

CONCLUSION

In tissue culture studies enrichment of the culture medium with 2,4-D creates a genomic flux causing dedifferentiation of the tissues put in culture. Following redifferentiation useful variations could be generated. Most of these variations are heritable and contribute to the widening of the gene. In this study this potential has been fully exploited in both rice and wheat which are important cereals of Pakistan.

REFERENCES

Hill, G.P. (1967). Multiple budding on carrot embryos arising in tissue culture. Nature. 215: 1098-1099.

Jacobsen, H.J. (1983). Biochemical mechanisms of plant hormone activity. In. hand book of Plant Cell Culture (D.A. Evan, W.R. Sharp, P.V. Ammirato and Y. Yamada eds). Collier Macmillan Publishers, London, PP. 672-695.

Murashige, T, and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Plant Physiol. 15:473-497.

Mackinnon, C; Gunderson, G. and Nabors, M.W. (1987). High frequency plant regeneration by somatic embryogenesis from calli of mature embryo explants of bread wheat (*Triticum aestivum*) and grain sorghum (*Sorghum bicolor*). In vitro cellul. Develop Bio. 23(6): 443-448.

Nabors, M.W. and Dykes, T.A. (1985). Tissue culture of cereal cultivars with increased salt, drought, and acid tolerance. In: Biotechnology in International Agricultural Research. Proceedings of the International Centre Seminar on International Agricultural Research Centres (IARCs) and Biotechnology, 23-27, April, 1984. Manila, Philippines PP. 121-138.