

Development of a Callogenic Protocol in Papaya (*Carica papaya* L.). 2. *In vitro* Grown Vegetative Explants

MUHAMMAD USMAN, B. FATIMA, MUHAMMAD J. JASKANI AND MUHAMMAD AZEEM IQBAL†

Department of Horticulture, University of Agriculture, Faisalabad-38040, Pakistan

†Nuclear Institute of Agriculture and Biology, Faisalabad-Pakistan

ABSTRACT

Different explants from *in vitro* grown seedlings of papaya were appraised for callogenesis on half strength organic and inorganic salts of Murashige and Skoog (MS) media. It was supplemented with 6% of sucrose and growth regulators like 2,4-D (2.5, 5.0 & 7.5 mg L⁻¹) and kinetin (0.5, 1.0 & 1.5 mg L⁻¹) for callogenesis, separately. Media for regeneration and proliferation consisted of MS media modified with NAA (1.0, 3.0 & 5.0 mg L⁻¹). Earliest callus induction was observed from stem cutting explant on kinetin at 1.5 mg L⁻¹ on 5th day of culture. Callus initiation revealed stem cutting as the best explant with 290% callus induction in all the treatments used. Maximum callus growth was noticed in leaf blade with midrib explant (1.1939 g) while Stem cutting showed earliest callus multiplication on NAA (5 mg L⁻¹) on 5th day of culture. Maximum callus multiplication was yielded by petiole explant derived callus (60% cultures) on NAA (3 mg L⁻¹). The protocol may help to propagate male and female plants swiftly by subsequent embryogenesis and organogenesis. It may further be contributive in developing synthetic seeds and transgenic plants of papaya.

Key Words: Callogenesis; Papaya; *In vitro*; 2,4-D; Kinetin; NAA

INTRODUCTION

A detailed introduction on the subject has already been given (Usman *et al.*, 2002). Briefly, several scientists have worked on development of callogenesis from tissues like hypocotyl (Fitch, 1993), lamina and midrib explants (Prahardini & Sudaryono, 1992), shoot tips (Yang & Ye, 1992) and petiole (Mosella & Iligaray, 1985); root, stem and leaf segments from *in vitro* grown seedlings (Mondal *et al.*, 1994), and suspension cultures (Ye *et al.*, 1993). Callus has successfully been induced on different media formulations supplemented with several different growth regulators (Litz *et al.*, 1983; Moore & Litz, 1984; Medora *et al.* 1984; Drew, 1987; Rojas & Kitto, 1991; Fitch, 1993; Mondal *et al.*, 1994; Jordan & Velozo, 1995). Induction of callus in media containing 2, 4-D has also been reported for many plants previously (Medora *et al.*, 1979; Kumar *et al.*, 1992; Yang & Ye, 1992; Fitch, 1993). Likewise, addition of kinetin to the media for callus induction has been supported (Jordan *et al.*, 1983; Mondal *et al.*, 1990; Jordan & Velozo, 1995). The contrary findings are with addition of NAA to MS media in *C. candamarcensis* (Jordan *et al.*, 1983), in pawpaw with ammonium nitrate (Medora *et al.*, 1979), BA (Yang & Ye, 1992) and NAA (Litz *et al.*, 1983; Jordan, 1989; Prahadini & Sudaryono, 1992), NAA and GA (Mosella & Iligaray, 1985), adenine sulphate and CH (Winnaar, 1987), GA (Mosella & Iligaray, 1985), and NAA in combination with zeatin and benzyl adenine (Mondal *et al.*, 1994).

This paper reports results of the 2nd study of the series of experiments conducted on the evaluation of various kinds of explants and growth regulators for callogenesis. The findings on development of a callogenic protocol in

papaya (*Carica papaya* L.) using *in vivo* grown vegetative explants have already been reported (Usman *et al.*, 2002). Therefore, this paper describes the results of a similar study using *in vitro* grown vegetative explants.

MATERIALS AND METHODS

The basal medium contained half strength organic and inorganic salts of Murashige and Skoog (1962) supplemented with 6% of sucrose. The medium pH was adjusted to 5.7 and gelrite (1.6%) was added in the media as solidifying agent. Media was autoclaved for 15 min at 121°C under 1.5 Kg/cm² pressure. For callogenesis, basal media was supplemented with 2, 4-D (2.5, 5.0 & 7.5 mg L⁻¹) and kinetin (0.5, 1.0 & 1.5 mg L⁻¹), separately. Regeneration and proliferation media contained MS modified with NAA (1.0, 3.0 & 5.0 mg L⁻¹). Explant types employed for callogenesis were leaf blade (0.5 cm²), leaf blade with midrib (0.5 cm²), petiole (0.5 cm), leaf blade with midrib plus petiole (0.5 cm³), stem cutting (0.5 cm), primary root (0.5 cm), secondary root (0.5 cm), root tip (0.5 cm), and callus/callogenic mass (0.5 cm²). Explants were surface disinfested by submerging in 70% alcohol plus one to two drops of Tween 20 as surfactant (one minute) and in 0.5% NaOCl (two to three minutes) followed by 3-5 washings with autoclaved deionized water.

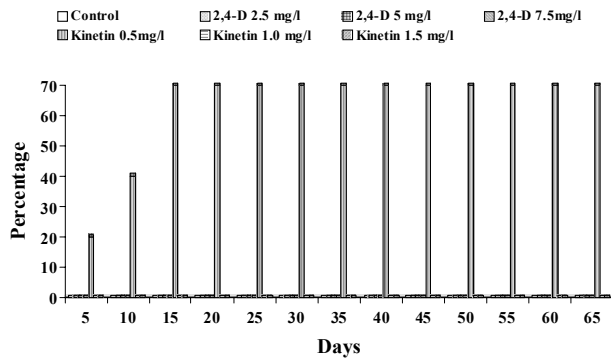
Explants were excised and cultured in the callogenic media and were kept in growth room at temperature 25 ± 2°C and fluorescent light intensity of 2500 lux. Callus generated was weighed on fresh weight basis and subcultured on the regeneration and proliferation media. Data were collected as: callus induction (days), callus

initiation (%), callus growth (g), callus multiplication (days) and callus multiplication (%).

RESULTS AND DISCUSSION

Time span for callus induction (days). Leaf blade explant induced no callus on any treatment. In leaf blade with midrib explant, callus induction was found only on kinetin (0.5 mg L⁻¹) on 5th day of culture (Fig. 1). The results are in line with Litz *et al.* (1983) as far as the explant and media applied (half strength MS medium) are concerned. The results are further intensified by Winnaar (1987) and Mondal *et al.* (1994) as the explant employed is concerned.

Fig. 1. Time span for callus induction in leaf blade with midrib explant of papaya



Petiole explant generated callus on media containing kinetin (0.5 mg L⁻¹) on 5th day (Fig. 2). Results exhibited deviation from the findings of Yang and Ye (1992) who induced callus on 2, 4-D and BA while our results showed no callus induction on any level of 2, 4-D. The logic seems as if difference might be due to variation in the strength of MS media and the concentration of 2, 4-D employed. Stem cutting induced maximum callus on kinetin (1.5 mg L⁻¹) in minimum duration (5 days) ensued by 2, 4-D at 2.5 mg L⁻¹ (Fig. 3). The findings are controversial to the results of

Fig. 2. Time span for callus induction in petiole explant of papaya

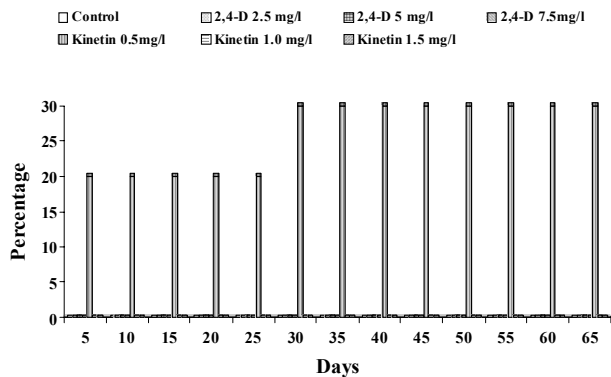
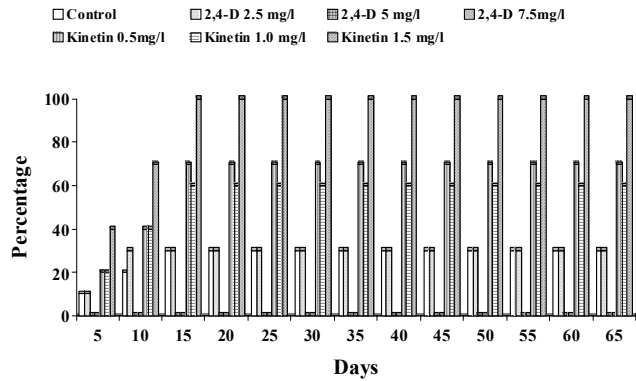
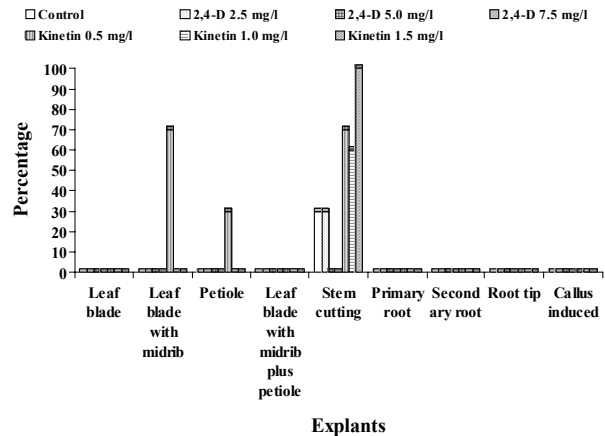


Fig. 3. Time span for callus induction in stem cutting explant of papaya



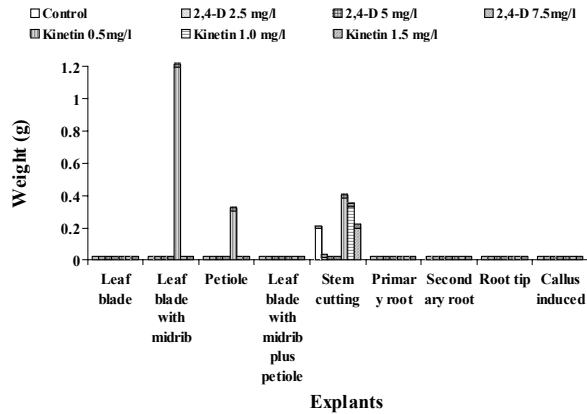
Mondal *et al.* (1994). They obtained maximum callus from root explants on half strength MS media with IBA and kinetin. The results do agree with the findings of Winnaar (1987) as far as the explant used is concerned while the basal media formulations, growth regulators and the cultivar employed (Sunrise Solo) were different. Callus induction was observed in 3-4 weeks of culture. Leaf blade with midrib plus petiole explant yielded no callus on any media. Primary root, secondary root and root tip also remained unresponsive. Callus derived from seedlings when cultured on callus proliferation media showed no multiplication. Regarding the media formulations and the growth regulators Kumar *et al.* (1992) found 2, 4-D as the best growth regulator for callus induction. Fitch (1993) induced embryogenic callus on half strength MS medium supplemented with 2, 4-D. These findings do agree with the results.

Fig. 4. Effect of growth hormones on callus initiation (%) of papaya explants



Callus initiation (%). Among the explants used, stem cutting proved as the best explant on the basis of total callus initiation (290%) ensued by leaf blade with midrib (70%) and petiole yielding 30% callus (Fig. 4). The findings in stem cutting are supported by Winnaar (1987). The results

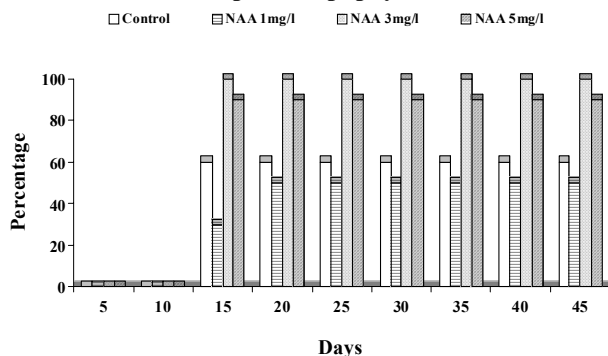
Fig. 5. Effect of growth hormones on callus growth of papaya explants



are incompatible to Mondal *et al.* (1994), might be due to difference in media formulations employed. In leaf blade, the results obtained are supported by Litz *et al.* (1983). Further justification is provided by Winnaar (1987) and Mondal *et al.* (1994). In case of petiole, results are controversial to findings of Mondal *et al.* (1994) for growth regulators used and Yang and Ye (1992) with respect to callus initiation. Concerning the treatments employed (170%) for callus initiation for all the explants used followed by kinetin 1.5 mg L⁻¹ (100%) and 1.0 mg L⁻¹ (60%). 2, 4-D (2.5 mg L⁻¹) and control induced similar percentage of callus (30%). Analysis of cumulative effect of growth regulators at various levels depicted extremely higher response of the explants for callus initiation on media modified with kinetin (330%) than 2, 4-D with 30% callus initiation, only (Fig. 4). It is verified by the findings of Jordan *et al.* (1983) and Mondal *et al.* (1990). Kumar *et al.* (1992) and Fitch (1993) further strengthened the better performance of 2, 4-D on the other growth regulators except kinetin.

Callus growth (g). Leaf blade with midrib explant produced maximum (1.1939 g) callus followed by stem cutting (1.1238 g) and petiole (Fig. 5). Little is known about the callus growth in terms of weight. Further standardisation

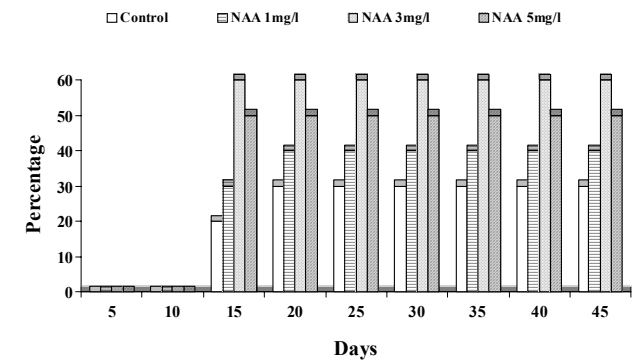
Fig. 6. Time span to induce callus multiplication in leaf blade with midrib explant of papaya



is desired for verification. Treatment comparison depicted kinetin (0.5 mg L⁻¹) as the best treatment (1.8843 g) ensued by kinetin (0.3327 g) at 1.0 mg L⁻¹. Among growth regulators, utilised kinetin (2.4163 g) was better than 2, 4-D (0.0124 g) with respect to total callus growth (Fig. 5). The results are supported by the findings of Jordan *et al.* (1983) and Modal *et al.* (1990) who reported better performance of kinetin.

Time span to induce callus multiplication (days). In leaf blade with midrib explant, all the treatments initiated callus multiplication on 15th day of subculture (Fig. 6). Petiole yielded callus multiplication on 15th day of subculture in all treatments employed (Fig. 7). Stem cutting showed earliest callus multiplication on NAA (5 mg L⁻¹) ensued by NAA (1 & 3 mg L⁻¹) and control (Fig. 8). Results are contrary to the findings regarding the basal media application as MS media (Mondal *et al.*, 1990; Kumar *et al.*, 1992), modified White's

Fig. 7. Time span to induce callus multiplication in petiole explant of papaya



media with coconut water (Medora *et al.*, 1979; 1984) and De Fossard medium (Drew, 1987). The results vary regarding the growth regulators employed as NAA and BA by Mondal *et al.* (1990) and kinetin by Kumar *et al.* (1992).

Callus multiplication (%). Leaf blade and leaf blade with midrib plus petiole, primary and secondary roots, root tip and callus derived from seedlings showed no response on any treatment. Petiole produced maximum callus (60%

Fig. 8. Time span to induce callus multiplication in stem cutting explant of papaya

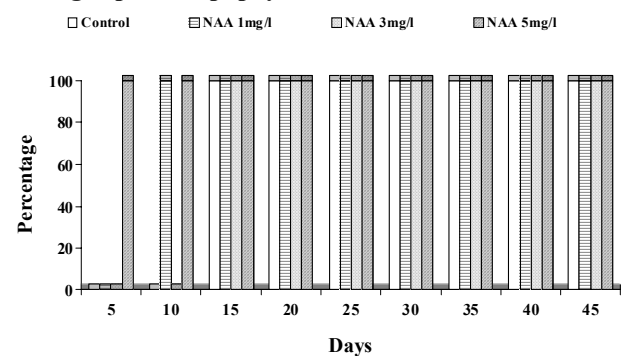
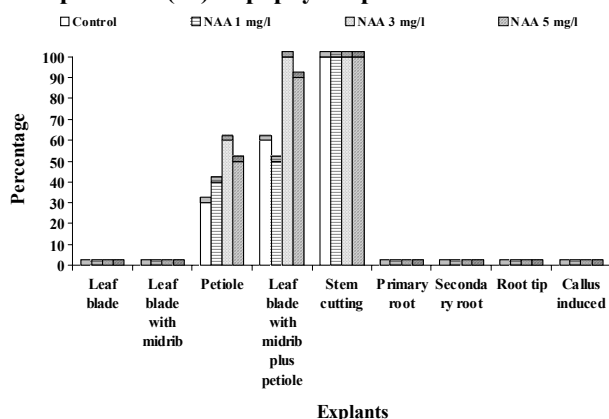


Fig. 9. Effect of growth hormones on callus multiplication (%) of papaya explants

cultures) on NAA (3 mg L⁻¹) followed by 50, 40 and 30% on NAA (5 & 1 mg L⁻¹) and control, respectively. Leaf blade with midrib derived callus generated maximum multiplication (100% cultures) on NAA (3 mg L⁻¹) ensued by 90, 60 and 50% on NAA (5 mg L⁻¹), control and NAA (1 mg L⁻¹), respectively. Stem cutting gave callus multiplication in 100% cultures on all treatments (Fig. 9). Maximum rate of total callus multiplication on all treatments was yielded by stem cutting (400%) followed by leaf blade (300%) and petiole (180%). NAA (3 mg L⁻¹) proved the best level with 260% of total callus multiplication ensued by NAA 5 mg L⁻¹ (240%). NAA (1 mg L⁻¹) and control gave similar percentage i.e., 190% (Fig. 9). The findings of Mondal *et al.* (1990) are antagonistic to our results as they have reported callus growth on NAA and BA. Similarly, Kumar *et al.* (1992) reported callus growth on kinetin.

The comparison of various explants, obtained from *in vitro* raised seedlings of papaya (*Carica papaya* L.) revealed that stem cutting/nodal culture might be much better explant for callus induction on the MS media modified with kinetin as the growth regulator. The callogenic protocol development may be helpful to propagate male and female plants swiftly by subsequent embryogenesis and organogenesis. It may further be contributive in developing synthetic seeds and transgenic plants of papaya.

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