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Short Communication

Tissue Culture Studies in Blackgram [*Vigna mungo* (L.) Hepper]

Sandeep Kaushish* and V.L. Mathur
Department of Plant Breeding & Genetics, Rajasthan College of Agriculture, Udaipur 313 001, India

Blackgram [*Vigna mungo* (L.) Hepper], belonging to family Fabaceae, is a native of India and originated from tribe Phaseolus. In India it occupies an area of 3.15 m ha with a production of 1.49 m tonnes (Asthana and Chaturvedi, 1999). Productivity of blackgram is not only low, but also remained stagnant during the last few years, due to lack of adequate breeding efforts to improve the yield. Existence of genetic variability is the basic prerequisite for selection and improvement of any crop plant. However, genetic variability in Vigna species is low due to self-pollinating nature of the crop. Besides, conventional methods, tissue culture has great potential in crop improvement, as these approaches create genetic variability and diversity (Newell *et al.*, 1984; Amato, 1985). The present study was aimed at determining the best source of explants for maximum callus induction and regeneration so that the technique can be employed for the development of somaclonal variants.

Seeds of blackgram cv. Krishna were surface-sterilized with 70% ethanol for 30 seconds and 0.1% mercuric chloride solution for five minutes. After three washings with distilled water in ultraviolet cabinet, these seeds were grown on MS medium (Murashige and Skoog, 1962). The MS and B5 media (High Media Laboratory Pvt. Ltd., Bombay; Gamborg *et. al.*, 1968) were supplemented with 2, 4-D or kinetin (Kn), individually or in combination (Table 1). The pH of the media MS was adjusted to 5.8 and that of B5 to 5.5 prior to autoclaving. The flasks containing media were tightly plugged with non-absorbent cotton and covered with aluminium foil and autoclaved at 1.1 kg cm^{-2} pressure, for 20 minutes. The explants (cotyledon and embryonal axis) taken from in vitro germinated seedlings (five-day-old) were inoculated on MS or B5 media in conical flasks and were transferred to a growth chamber maintained at 26±2°C. Callus tissues were harvested from each treatment after 30 days for recording fresh and dry weights.

Of the two phytohormones supplemented in media, 2, 4-D was found promising compared to Kn in producing more fresh and dry weights of callus irrespective of the media (Table 1). Similarly, explants (cotyledon and embryonal axis) also exhibited significant differences with regard to callus weight between media viz., MS and B5. Narcisa and Hattori (1995) also established the importance of medium used in callus induction.

* Central Arid Zone Research Institute, Jodhpur 342003, India.

Table 1. Mean fresh and dry callus weights after 30 days of inoculation using cotyledon and embryonal explants of blackgram on MS and B5 media

Treatment	Cotyledon explant				Embryonal axis explant			
	Fresh callus weight (g)		Dry callus weight (g)		Fresh callus weight (g)		Dry callus weight (g)	
	MS	B_5	MS	B_5	MS	B_5	MS	B_5
Kinetin 0.1 mg L^{-1}	0.1325	0.0212	0.0143	0.0107	0.0385	0.0520	0.0024	0.0039
Kinetin 0.5 mg L^{-1}	0.1699	0.0253	0.0152	0.0109	0.0383	0.0562	0.0041	0.0043
2, 4-D 0.5 mg L^{-1}	0.1825	0.0355	0.0156	0.0110	0.0459	0.0580	0.0065	0.0052
2, 4-D 1.0 mg L^{-1}	0.1974	0.0447	0.0165	0.0132	0.0512	0.0600	0.0095	0.0061
2, 4-D 0.5 mg L^{-1} + Kinetin 0.1 mg L^{-1}	0.1389	0.0265	0.0146	0.0125	0.5567	0.0580	0.0200	0.0042
2, 4-D 0.5 mg L^{-1} + Kinetin 0.5 mg L^{-1}	0.1585	0.0477	0.0158	0.0128	0.5901	0.0591	0.0262	0.0047
2, 4-D 1.0 mg L^{-1} + Kinetin 0.1 mg L^{-1}	0.1870	0.0518	0.0159	0.0135	0.6333	0.0630	0.0344	0.0065
2, 4-D 1.0 mg L^{-1} + Kinetin 0.5 mg L^{-1}	0.1990	0.0578	0.0165	0.0148	0.6507	0.0675	0.0342	0.0072
S EM ±	0.0030	0.0023	0.0002	0.0002	0.0482	0.0013	0.0019	0.0003
CD at 5%	0.0063	0.0048	0.0004	0.0005	0.1022	0.0028	0.0037	0.0006

Irrespective of explants and media used, callus weight increased with increasing concentration of growth regulators. These findings are in conformity with the observations made by Umadevi and Bai (1995) in case of *V. radiata* and *V. glabrescense*. Among the four hormonal combinations,the treatment 1.0 mg L^{-1} 2, 4-D + 0.5 mg L^{-1} Kn proved better over other treatments for callus weight. These observations are in agreement with that of Pandey and Bansal (1989) reported for *V. sinensis*. In general, higher callus weight was recorded when media was supplemented with hormonal combination instead of individual hormone. Graceo *et al.* (1984) explained that one hormone enhanced the level of another by promotion of biosynthesis or inhibition of degenerative metabolism.

It is concluded from the present investigation that regeneration was achieved from both the explants cultured on MS medium. Maximum frequency of regeneration (71.4%) was noticed at 0.1 mg L^{-1} Kn with cotyledon explant, while it was only 33.3% in case of treatment 1.0 mg L^{-1} 2, 4-D + 0.1 mg L^{-1} Kn with embryonal axis explant. The plantlets of embryonal axis explant survived only for 10-15 days, whereas those from cotyledon explant survived upto one month after transferring them to the pot. The plantlets recorded a height of 10-15 cm with 3-4 leaves and few roots. There is, however, need to further refine the protocols to harden seedlings and prolong their ability until maturity.

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