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AN EFFICIENT PROTOCOL FOR CALLOGENESIS OF POTATO CULTIVARS (SIFRA & RODEO)

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ABSTRACT

The present study was conducted to standardize an efficient protocol for *in vitro* callogenesis of two potato cultivars (Sifra and Rodeo) from four explants i.e. leaf, node, internode and tubers. MS medium was supplemented with different concentrations of auxins (2,4-D and NAA) cytokinins (Kin and BAP) either alone or in combination with each other. The best degree of callus induction (96%) was observed from tubers of cultivar Sifra on MS medium containing 3.0 mg/L of 2,4-D. Regeneration of plantlets was also observed from tubers and tuberous callus of cultivar Sifra on MS medium supplemented with 3.0 mg/L of 2,4-D.

Keywords: Callogenesis, Potato cultivars, Sifra, Rodeo

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) belongs to nightshade family *Solanaceae* and is considered as 4th most important tuberous vegetable crop in Asia and Pakistan. It remains an essential crop in Europe (especially eastern and central Europe) where per capita production is still highest in the world. Just over 2/3rd of the global production of potato is eaten directly by humans with the rest being fed to animals or used to produce starch.

It contains some glycoalkaloids (like solanine and chaconine) but supplies at least 12 essential vitamins, minerals, proteins, carbohydrates and iron. More than 300 million metric ton of potatoes are produced in the world (Hermansen *et al*., 2012) but yield of potato (219296 Hg/Ha) in Pakistan is too low as compared to other potato growing countries of the world (FAO, 2013).

The low yield is mainly due to the lack of true to type, free and premium quality seed potatoes. Different types of biotic (Fungal and viral diseases) as well abiotic stress (heavy metal and salinity) have significant role in low production of potato (Gichner *et al*., 2008).

As the population of the world is increasing at a quite rapid rate, there is a dire need to enhance the yield and production of major staple food crops in order to fulfil the food requirements of a huge population. It is possible only through efficient screening of resistant potato cultivars. Field screening is laborious, time taking, and season dependent as well while *in vitro* screening through plant tissue culture techniques is a rapid and interesting way.

Among various tissue culture techniques, Callogenesis is an essential step for study of various physiological phenomena including resistance against various biotic and abiotic stresses. So, the present research work was carried out to establish an efficient protocol for callogenesis of potato.

2. MATERIALS AND METHODS

Explants (leaf, node, internode and tubers) of potato cultivars were taken from fields of University of Sargodha. For surface sterilization, explants were first washed with running tap water for 10 minutes. After that explants were treated with household detergent followed by three rinses with running tap water. Then, they were dried with blotting paper to remove all traces of tap water. One drop of Tween-20 was used to reduce the surface tension of explants and after 3 rinses with double distilled water, sterilization was followed by the use of commercial bleach which contains 15% sodium hypochlorite (NaOCl). Finally, explants were cultured on MS medium supplemented with various concentrations of auxins (2,4-D and NAA) and cytokinins (BAP and Kinetin) for callus induction.

3. RESULTS AND DISCUSSION

Data presented in table 1 and 2 depicts that four different types of explants (node, internode, leaves and tubers) were used to study the effect of different growth regulators (NAA, 2,4-D, BAP) on callus induction in potato cv. *Sifra and Rodeo*.

In present study MS medium was supplemented with various concentrations (1-4 mg/L) of 2,4-D and 96% callus induction response was observed from tubers of Sifra at 3.0 mg/L of 2,4-D followed by 92% from internodes, 84% from nodes and 72% leaves (Table,1; Fig. 1a, b, c and d).

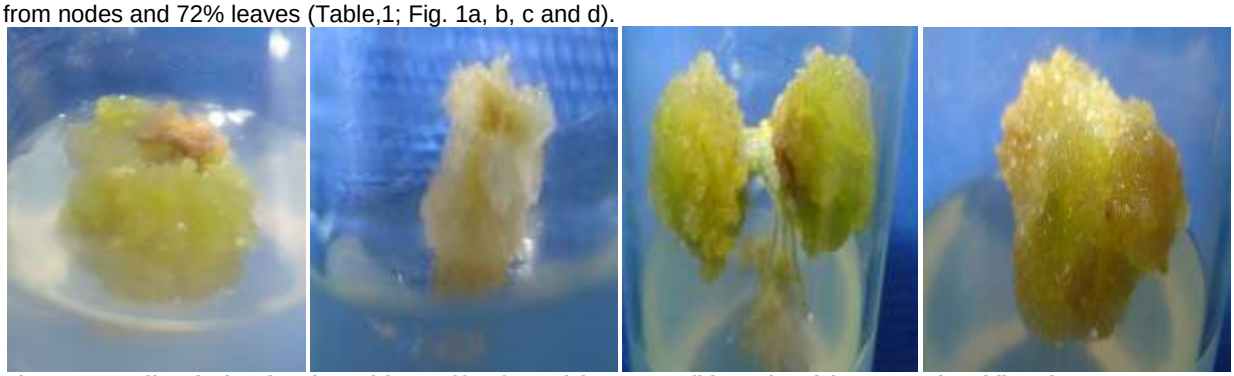


Figure 1. Callus induction in cultivar Sifra from (a) Leaves (b) Nodes (c) Internodes (d) Tubers on MS medium supplemented with 3.0 mg/L of 2,4-D

Similarly maximum callus induction was observed at same concentration of 2,4-D in case of cultivar Rodeo. Rate of callus induction was 88% in case of tubers followed by 84% from internodes, 80% from nodes and 68% from leaves (Table, 2; Fig. 2 a, b, c and d). Findings of Shirin *et al.,* (2007) and Khadiga *et al.,* (2009) are in line to our results who reported 3.0 mg/L 2,4-D most effective auxin concentration for callus induction in potato cultivars.

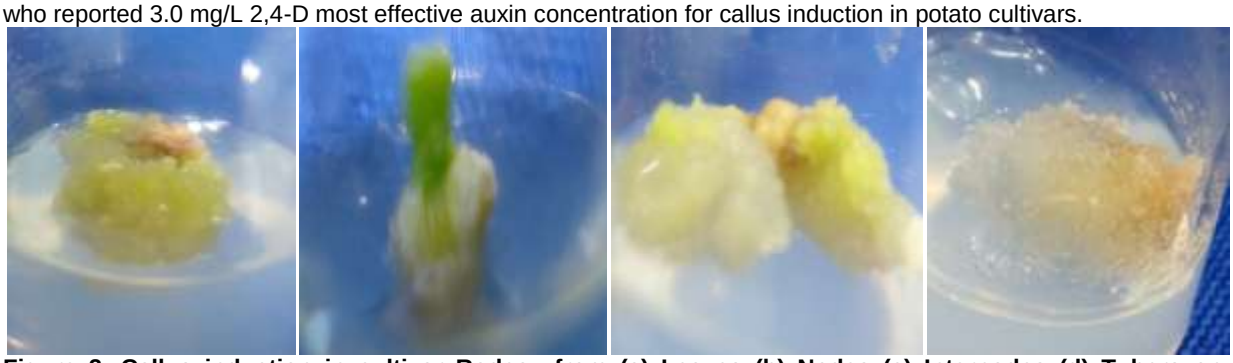
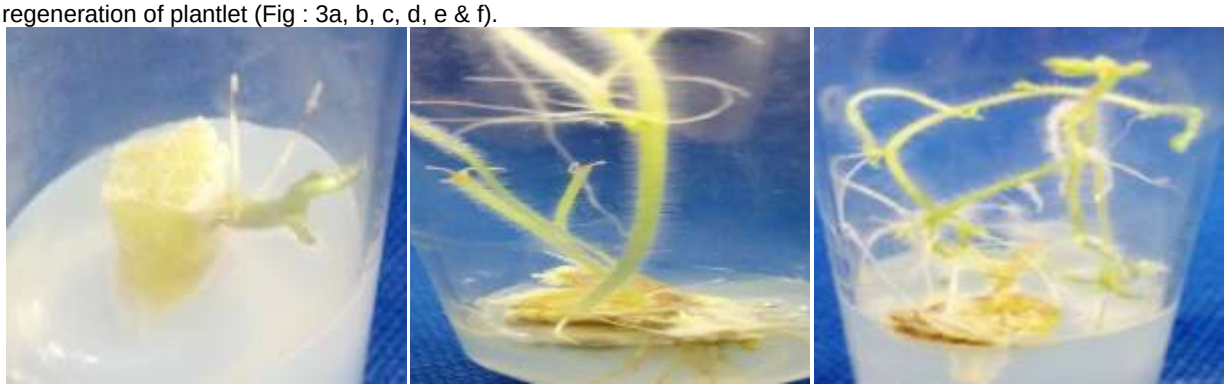


Figure 2. Callus induction in cultivar Rodeo from (a) Leaves (b) Nodes (c) Internodes (d) Tubers on MS medium supplemented with 3.0 mg/L of 2,4-D

No scientist have reported regeneration on MS medium supplemented with 2,4-D. In present study the phenomena of regeneration from tuber and tuberous callus of cultivar Sifra was observed may be due to variation in culture conditions, genotype of plant, type of explants or high endogenous level of cytokinins which may causes regeneration of plantlet (Fig : 3a, b, c, d, e & f).



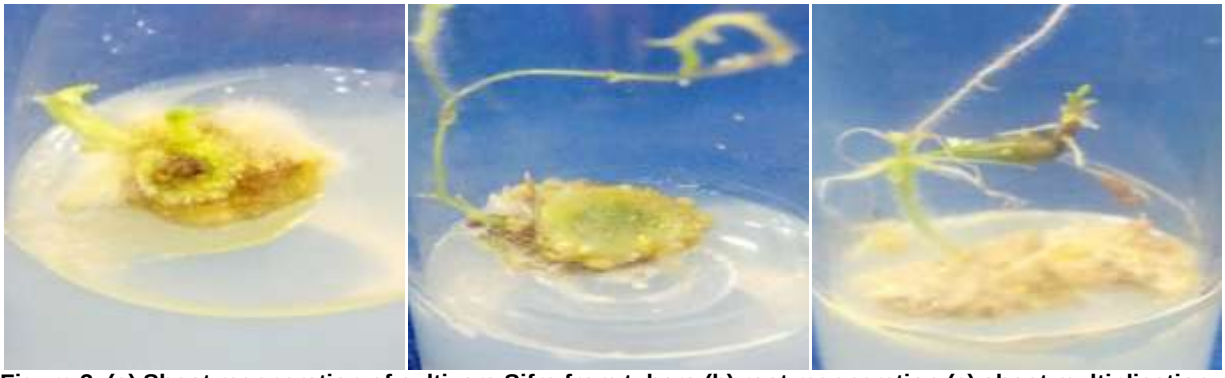


Figure 3. (a) Shoot regeneration of cultivars Sifra from tubers (b) root regeneration (c) shoot multiplication (d)shoot regeneration from tuberous callus of Sifra (e) shoot elongation(f) tuber formation on MS medium supplemented with 3.0 mg/L of 2,4-D

Many researchers like Castillo *et al*., (1998); Shirin *et al*., (2007); Khadiga *et al*., (2009) reported that 2,4-D by itself or in combination with cytokinin (BAP, KIN) is very effective to enhance callus induction and maintenance. Therefore, in present study MS medium was supplemented with different concentrations of 2,4-D, NAA in combination with KIN, but maximum degree of callus induction was observed from tubers of both cultivars under study on MS medium supplemented with a combination of 3.0 mg/L 2,4-D + 0.5 mg/L NAA and 1.5 mg/L KIN. Whereas findings of Khalkho and Sahu (2012) show contradiction with our results because they reported maximum callus induction from internodes instead of tubers. This contradiction in results is might be due difference in composition of MS medium which was supplemented with coconut milk. As the coconut milk has high mitotic activity and therefore it enhances the activity of 2,4-D and rate of cell division in the explant inoculated on MS medium.

Data presented in table 1 and 2 depicts that 88% and 84% callus induction was observed from leaves and internodes of Sifra and Rodeo on MS medium supplemented with a combination of 2,4-D (1.0 mg/L) and Kinetin (0.25 mg/L) respectively. In present study similar results were obtained from another combination of BAP and NAA. These results are in complete coherence with the findings of Sattar *et al.* (2011) who also reported best degree of callus induction from same combination and same type of explants. While a contradiction with other concentrations and combinations of growth regulators under examination was noticed here. Because best degree of callus induction was obtained from tubers instead of leaves of both cultivars (Sifra and Rodeo) on MS medium supplemented with all combinations of auxins and cytokinins except BAP + NAA and 2,4-D + Kinetin. This contradiction is may be due to variation in type of explants that show specific response in specific composition of media because plant genes not only involved in plant growth and development but they also govern the inheritance of callus growth (Turhan, 2004). Therefore different scientists have reported maximum callus induction from different types of explants in case different cultivars and different medium composition (Steward and Caplin, 1951).

Table 1: Effect of Different Concentrations and Combinations of Various Growth Regulators (Auxins and Cytokinins) on Callus Induction in Potato cv. Sifra

MS Media + Phytohormone	Concentration (mgL^{-1})	Leaf		Node		Internode		Tuber	
		Mean No. of Vials Showing Callus Induction	Rate of Callus Induction (%)	Mean No. of Vials Showing Callus Induction	Rate of Callus Induction (%)	Mean No. of Vials Showing Callus Induction	Rate of Callus Induction (%)	Mean No. of Vials Showing Callus Induction	Rate of Callus Induction (%)
2,4-D	**1**	1.8 ± 0.33^{fg}	36	2.4 ± 0.35^{cd}	48	2.4 ± 0.35^{d}	48	2.6 ± 0.21^{ef}	52
2,4-D	**2**	2.4 ± 0.21^{cdefg}	48	2.8 ± 0.33^{abcd}	56	3.2 ± 0.33^{abcd}	64	3.4 ± 0.45^{bcdef}	68
2,4-D	**3**	3.6 ± 0.21^{abc}	72	4.2 ± 0.33^{a}	84	4.6 ± 0.21^{a}	92	4.8 ± 0.17^{a}	96
2,4-D	**4**	3 ± 0.28^{bcdef}	60	3.6 ± 0.53^{abcd}	72	4.4 ± 0.35^{ab}	88	4.4 ± 0.21^{ab}	88
2,4-D+KIN	**0.5+0.25**	3.2 ± 0.43^{abcde}	64	2.2 ± 0.33^{d}	44	3.2 ± 0.43^{abcd}	64	2.4 ± 0.21^{f}	48
2,4-D+KIN	**1+0.25**	4.4 ± 0.21^{a}	88	3.4 ± 0.45^{abcd}	68	4 ± 0.48^{abc}	80	3.8 ± 0.43^{abcde}	76
2,4-D+KIN	**2+0.25**	4 ± 0.28^{ab}	80	2.8 ± 0.17^{abcd}	56	3.6 ± 0.45^{abcd}	72	3 ± 0.48^{cdef}	60
2,4-D+KIN	**3+0.25**	3.4 ± 0.21^{abcd}	68	2.6 ± 0.21^{bcd}	52	3 ± 0.4^{bcd}	60	2.8 ± 0.33^{def}	56
2,4-D+NAA+KIN	**1+0.5+1.5**	3.2 ± 0.43^{abcde}	64	2.8 ± 0.59^{abcd}	56	3.2 ± 0.33^{abcd}	64	3.6 ± 0.21^{abcde}	72
2,4-D+NAA+KIN	**2+0.5+1.5**	2.4 ± 0.21^{cdefg}	48	3.8 ± 0.52^{abc}	76	4 ± 0.4^{abc}	80	4.2 ± 0.17^{abc}	84
2,4-D+NAA+KIN	**3+0.5+1.5**	3.6 ± 0.53^{abc}	72	4 ± 0.4^{ab}	80	4.4 ± 0.35^{ab}	88	4.6 ± 0.21^{ab}	92
2,4-D+NAA+KIN	**4+0.5+1.5**	2 ± 0.28^{efg}	40	3 ± 0.28^{abcd}	60	3.2 ± 0.33^{abcd}	64	3.6 ± 0.21^{abcde}	72
2,4-D+BAP	**1+0.5**	1.8 ± 0.43^{fg}	36	3 ± 0.4^{abcd}	60	3.6 ± 0.45^{abcd}	72	3.8 ± 0.33^{abcde}	76
2,4-D+BAP	**1+1**	2 ± 0.48^{efg}	40	3.6 ± 0.45^{abcd}	72	4 ± 0^{abc}	80	4.2 ± 0.33^{abc}	84
2,4-D+BAP	**2+2**	2.8 ± 0.33^{bcdef}	56	4 ± 0.48^{ab}	80	4.4 ± 0.21^{ab}	88	4.4 ± 0.35^{ab}	88
2,4-D+BAP	**3+1**	1.4 ± 0.21^{g}	28	3.2 ± 0.17^{abcd}	64	3.6 ± 0.35^{abcd}	72	4 ± 0.4^{abcd}	80
NAA+BAP	**0.5+0.5**	1.8 ± 0.33^{fg}	36	2.4 ± 0.21^{cd}	48	2.8 ± 0.52^{cd}	56	3 ± 0.48^{cdef}	60
NAA+BAP	**1+0.5**	2.2 ± 0.33^{defg}	44	2.8 ± 0.33^{abcd}	56	3.2 ± 0.52^{abcd}	64	3.4 ± 0.21^{bcdef}	68
NAA+BAP	**1+1**	3.4 ± 0.45^{abcd}	68	4 ± 0.56^{ab}	80	4.2 ± 0.17^{abc}	84	4.4 ± 0.21^{ab}	88
NAA+BAP	**1.5+0.5**	2.8 ± 0.33^{bcdef}	56	3.2 ± 0.52^{abcd}	64	3.6 ± 0.35^{abcd}	72	3.8 ± 0.33^{abcde}	76
LSD	**……**	**1.09**	**…**	**1.27**	**…**	**1.18**	**…**	**1.01**	**…**

Mean followed by different letters in the same column differ significantly at p= 0.05 according to Duncan's new multiple range test

Table 2: Effect of Different Concentrations and Combinations of Various Growth Regulators (Auxins and Cytokinins) on Table 2: Effect of Different Concentrations and Combinations of Various Growth Regulators (Auxins and Cytokinins) on Callus Induction in Potato cv. Rodeo

MS Media + Growth Regulator	Concentration (mgL^{-1})	Leaf		Node		Internode		Tuber	
		Mean No. of Vials Showing Callus Induction	Rate of Callus Induction (%)	Mean No. of Vials Showing Callus Induction	Rate of Callus Induction (%)	Mean No. of Vials Showing Callus Induction	Rate of Callus Induction (%)	Mean No. of Vials Showing Callus Induction	Rate of Callus Induction (%)
2,4-D	**1**	1.4 ± 0.35^{gh}	28	2.0 ± 0.28^{e}	40	2.2 ± 0.17^{d}	44	2.4 ± 0.21^{de}	48
2,4-D	**2**	2 ± 0.28^{defg}	40	2.6 ± 0.35^{cde}	52	2.8 ± 0.33^{bcd}	56	3.0 ± 0.28^{bcde}	60
2,4-D	**3**	3.4 ± 0.35^{abc}	68	4.0 ± 0.4^{a}	80	4.2 ± 0.17^{a}	84	4.4 ± 0.21^{a}	88
2,4-D	**4**	2.8 ± 0.33^{bcdef}	56	3.4 ± 0.21^{abcd}	68	3.8 ± 0.33^{abc}	76	4.0 ± 0.0^{abc}	80
2,4-D+KIN	**0.5+0.25**	2.8 ± 0.33^{bcdef}	56	2 ± 0.28^{e}	40	2.6 ± 0.21^{cd}	52	2.2 ± 0.33^{e}	44
2,4-D+KIN	**1+0.25**	4.2 ± 0.33^{a}	84	2.8 ± 0.33^{bcde}	56	3.2 ± 0.17^{abcd}	64	3 ± 0.4^{bcde}	60
2,4-D+KIN	**2+0.25**	3.8 ± 0.33^{ab}	76	2.4 ± 0.21^{de}	48	3 ± 0.4^{abcd}	60	2.8 ± 0.33^{cde}	56
2,4-D+KIN	**3+0.25**	3.2 ± 0.43^{abc}	64	2 ± 0.28^{e}	40	2.8 ± 0.17^{bcd}	56	2.6 ± 0.21^{de}	52
2,4-D+NAA+KIN	**1+0.5+1.5**	3.0 ± 0.28^{bcd}	60	3 ± 0.28^{abcde}	60	3 ± 0.48^{abcd}	60	3.4 ± 0.21^{abcd}	68
2,4-D+NAA+KIN	**2+0.5+1.5**	2.0 ± 0.28^{defgh}	40	3.8 ± 0.33^{ab}	76	4 ± 0.28^{ab}	80	4 ± 0.4^{abc}	80
2,4-D+NAA+KIN	**3+0.5+1.5**	2.8 ± 0.33^{bcdef}	56	4 ± 0.28^{a}	80	4 ± 0.4^{ab}	80	4.4 ± 0.21^{a}	88
2,4-D+NAA+KIN	**4+0.5+1.5**	1.8 ± 0.33^{efgh}	36	2.6 ± 0.21^{cde}	52	3 ± 0.4^{abcd}	60	3.2 ± 0.43^{abcde}	64
2,4-D+BAP	**1+0.5**	1.4 ± 0.35^{gh}	28	3 ± 0^{abcde}	60	3.2 ± 0.17^{abcd}	64	3.6 ± 0.21^{abcd}	72
2,4-D+BAP	**1+1**	1.8 ± 0.17^{efgh}	36	2.8 ± 0.17^{bcde}	56	3 ± 0.4^{abcd}	60	4 ± 0.28^{abc}	80
2,4-D+BAP	**2+2**	2.6 ± 0.21^{cdef}	52	3.2 ± 0.17^{abcd}	64	3.4 ± 0.45^{abcd}	68	4.2 ± 0.33^{ab}	84
2,4-D+BAP	**3+1**	1.2 ± 0.17^{h}	24	3.0 ± 0.4^{abcde}	60	3.2 ± 0.17^{abcd}	64	3.6 ± 0.45^{abcd}	72
NAA+BAP	**0.5+0.5**	1.6 ± 0.21^{fgh}	32	2 ± 0.28^{e}	40	2.2 ± 0.33^{d}	44	2.8 ± 0.33^{cde}	56
NAA+BAP	**1+0.5**	1.8 ± 0.33^{efgh}	36	2.6 ± 0.21^{cde}	52	2.8 ± 0.33^{bcd}	56	3.2 ± 0.52^{abcde}	64
NAA+BAP	**1+1**	2.8 ± 0.33^{bcdef}	56	3.6 ± 0.21^{abc}	72	4 ± 0.4^{ab}	80	4.2 ± 0.33^{ab}	84
NAA+BAP	**1.5+0.5**	2.4 ± 0.21^{cdefg}	48	2.6 ± 0.35^{cde}	52	3.2 ± 0.17^{abcd}	64	3.4 ± 0.21^{abcd}	68
LSD	**……**	**0.97**	**…**	**0.88**	**…**	**1.00**	**…**	**1.00**	**…**

Mean followed by different letters in the same column differ significantly at p= 0.05 according to Duncan's new multiple range test

4. CONCLUSION

It is concluded from present study that 3 mg/L of 2,4-D in MS medium is best concentration for callogenesis of potato cultivars from tubers and internodal explants.

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