Pak. J. Bot., 50(5): 1871-1876, 2018.

EFFECT OF DIFFERENT GROWTH REGULATORS ON *IN VITRO* PROPAGATION OF *BRASSICA NAPUS* L.

SARWAT NAZ[1], MUHAMMAD FAHEEM SIDDIQUI[1]*AND SABOOHI RAZA[2]

[1]*Department of Botany, University of Karachi, Karachi-75270, Pakistan*
[2]*Department of Agriculture and Agribusiness Management, University of Karachi, Karachi-75270, Pakistan*
[*]*Corresponding author's email: mfsiddiqui@uok.edu.pk*

Abstract

A large amount of foreign exchange is expending to import the edible oil in Pakistan. The improvement of local oil producing varieties such as canola may play a vital role for better socio-economic condition of Pakistan. Plant tissue culture technique is one of the methods for crop improvement through somaclonal variation. The present study was conducted to evaluate the response of different explants including hypocotyl, leaf, node and cotyledon of canola (*Brassica napus* L.) on Murashige and Skoog medium supplemented with 2,4-D, NAA, IBA, BAP, 2,4-D+BAP at various concentrations. Each explant responds differently in the presence of phytohormones. The best callus was obtained at 0.1 mg/l 2,4-D with hypocotyl explants. The optimized calli was further shifted to different shooting media supplemented with BAP (0-3mg/l), without or with silver nitrate (5mg/l) and BAP (2mg/l) with or without Kinetin (0-3mg/l). The maximum number of shoots was formed on MS media containing 5mg/l silver nitrate and 2mg/l BAP. The elongated shoots were inoculated on different rooting media containing half MS media augmented with or without IBA at different concentrations. The maximum root regeneration was noticed on hormone free half MS media. The present study will be useful for indirect propagation of *Brassica napus,* which could be utilised for crop improvement of via somaclonal variation.

Key words: *Brassica napus* L, Callus induction, Regeneration, Auxins, Cytokinins.

Introduction

Brassica napus L. belongs to family Brassicaceae (Cruciferae) commonly known as rapeseed or Canola. It is found in countries with temperate climate or as a winter crop in subtropical climate (Vaughan and Geissler, 2009). *Brassica napus* was improved through the conventional breeding for low erucic acid (<2%) and glucosinolates (<30 umol/g) for human consumption in form of 'Canola'. Canola was identified as healthy oil producing crop with low level of saturated fatty acid (7%). It contains high level of oleic acid, linoleic acid, and alpha-linolenic acid, which are good for cardiovascular health (Lin *et al*., 2013). Animal feed and biodiesel may also be produced from canola (Ray *et al*., 2010). It also bears medicinal importance as it is used as embollient, diuretic and anticaterrhal (Khare, 2007). The oil of canola seed was also reported to cure gallstone (Khare, 2007).

Pakistan imports about 1.79 million tonnes of edible oil/ oil seeds that cost approximately US $ 1.37 billion (Pakistan Bureau of Statistics, 2015). *Brassica napus* L. is the second most important source of edible oil in Pakistan (Munir *et al*., 2008). Pakistan produced 207 thousand tonnes of rape seed in 2016 by cultivating it on an area of 226 thousand hectare. The world average rape seed production is 2.23tonnes/hectare. However in Pakistan the average production of rape seeds per unit hectare is only 0.8548 tonnes/hectare, since last twenty year. In 2016 only 0.9144 tonnes/hectare yields was produced (FAO statistics, 2018). The reduced yield potential of the cultivated varieties needs an improvement of this crop for better yield.

Plant tissue culture techniques offer an opportunity for the genetic improvement of the crop (Kharb *et al*., 2002). Plant tissue culture technique has been successfully utilized for the conservation of germplasm of endangered and rare species, production of pathogen free plants (Fay, 1992) and to induce variability in many crops (Larkin & Scowcroft, 1981; Jain *et al*., 1990; Katiyar, 1997 and Kharb *et al*., 2002). In *Brassica napus* L. various explants were successfully used for callus induction by different workers; stem was used as explants by Sharma & Gupt, 2012. Many researchers used hypocotyls for callus induction (Dubey & Gupta, 2014; Borjian & Arak, 2013; Burbulis *et al*., 2008; Munir *et al*., 2008; Qamarunnisa *et al.,* 2015), while cotyledon was used as explants by Chamandosti*et al*., 2006; nodal stem was used by Dubey & Gupta, 2014 and anther by Hassan *et al*., 1994.

The present work was carried out in order to establish an optimized protocol for callus induction of *Brassica napus* L. variety Hyola 401 using different explants. Hypocotyls, cotyledons, nodal stems and leaves were used as explants with different growth regulators and to regenerate plantlets through callus.

Material and Methods

Plant material: Seeds of *Brassica napus* L. variety Hyola 401 provided by ICI life Sciences, Sahiwal were used in the current study. The seeds were sterilized with 2.5 % sodium hypochlorite having 3 - 4 drops of tween-20 for 20 minutes and followed by rinsing several times with sterile distilled water (Qamarunnisa *et al.,* 2015). The seeds were inoculated on Murashige & Skoog (MS, 1962) basal medium and kept at 25±2°C under dark conditions for 2 days then transferred in growth chamber under 16-h light/8-h dark cycle. Different parts of the germinated seedlings were used as explants for callus induction. Hypocotyls and cotyledons were excised from 6-7 days old seedlings. Whereas nodal stems (0.3-0.5 cm) and leaves were excised from 27-30 days old seedlings.

Callus induction: For callus induction, different formulations of MS media with phytohormones were used. Eighteen formulation of callus induction media were prepared with MS mediumaugmented with various concentrations (0, 0.1, 0.5, 1, 1.5 and 2 mg/l)of 2,4-dichlorophenoxy acetic acid (2,4-D), Indole-3-butyric acid (IBA), 6-Benzylaminopurine (BAP), 1-Naphthalene acetic acid (NAA) alone.Other media formulations include the combinations of BAP and 2,4-D (Table 1). All media were augmented with 30g/l sucrose and solidified with 2.5g/l gelrite at pH 5.8. Explants including hypocotyls, cotyledons, nodal stems and leaves were inoculatedon different formulations of callus induction media in 10 replications. The cultured explants were kept at 25±2ºC temperature under dark.

Shoot regeneration and multiplication: Different regeneration medium was formulated by augmenting the MS medium with varying concentrations of BAP (0-3mg/l) with/ without silver nitrate (5mg/l) and also BAP (2mg/l) with/without kinetin (0-3mg/l). After 4weeks of callus induction, 0.7 gm of callus produced from the hypocotyls was transferred to shoot regeneration medium (Table 1). The calli were placed for photoperiodic regime16-h light/8-h dark cycle at 25±2°C. Each treatment contained 20 replicates. The shoots obtained from optimized shoot regeneration media were sub cultured in same media for shoot multiplication.

Root induction of regenerated shoots: *In vitro* regenerated and elongated shoot were separated and transferred to rooting media containing half strength MS media (hormone free) or augmented with IBA (0.1,0.5,1mg/l). Each treatment contained 10 replicates. Rooting was recorded after 30 days of culture.

Statistical analysis: Analysis of variance (ANOVA) was done using SPSS version 17. The effect of each treatment was evaluated in different explants and the significant difference by varying concentration was observed by Duncan Multiple Range Test (DMRT) at $p<0.05$. Same alphabets in one explant represent non-significant difference at $p<0.05$.

Result

Callus induction: Different explants i.e. hypocotyl, cotyledon, leaf and nodal stem were showed initial swelling of tissuesfollowed by callus formation within a week after inoculation on callus induction media. In hypocotyl and nodal stem, callus induction was started from the cut edges. However there was no callus formation when cotyledon, leaf and nodal stem were used as explants on hormone free medium, even after 48 days. Whereas, small amount of callus was formed from hypocotyls on hormone free medium.

Statistically different responses of callus induction were observed with different growth regulators and also with different explants at $p<0.05$. Callus formation was observed from the hypocotyls, cotyledons and from the nodal stem by the application of NAA (Fig. 1).Highest amount of white and transparent callus (0.52 g) was formed on hypocotyl, after 48 days. This callus also showed some adventitious roots along with callus.

Similarly, MS medium augmented with IBA also was not induced callus in any explants except in hypocotyls and nodal explant (Fig. 2). Nodal stem explant showed substantially lowest amount of callus only at 0.5 mg/l. The highest amount of transparent and white callus was observed in hypocotyl at 1mg/l of IBA (0.31 g) after 48 days of culture.

Different concentrations of 2, 4-D positively influences the weight of callus in all explants used. Substantially increased amount of callus was observed in all treatments as compare to control. In hypocotyl explant, the remarkable increase in weight of callus was observed at 0.1 mg/l of 2,4-D (Fig. 3a and b). Higher concentrations of 2,4-D (0.5 mg/l or above) inhibited the weight of callus in hypocotyl.In leaf explant the growth of callus was reduced by increasing the concentration of 2,4-D from 0.1 (Fig. 3a). Growth of callus was also reduced in callus obtained from cotyledon at higher concentration of 2,4-D (0.5 to 2mg/l); however in nodal explant calli was only obtained at 0.1 mg/l 2,4-D (Fig. 3a).

BAP was found to be effective for callus induction at all concentrations irrespective of the explants (Fig. 4). Hypocotyl showed the highest amount of callus at 1 mg/l BAP, whereas higher concentrations of BAP inhibited the growth of callus in hypocotyls culture. In cotyledons the highest amount of callus (0.48 g) was found at 1.5 mg/l of BAP. The treatment of leaf explants with BAP resulted in highest induction of soft brown callus (0.58 g) at 1mg/l concentration. Nodal explants induced callus was only found at 0.5mg/l concentration.

Table 1. Effect of different phytohormones and AgNO3 on callus regeneration of *Brassica napus* L. after 28 days of culture.

BAP Conc. (mg/l)	Kinetin Conc. (mg/l)	$AgNO_3$ Conc. (mg/l)	Mean weight of callus (g)*	Number of shoots Mean±SE	Length of shoots Mean±SE	Number of leaves Mean±SE
0	0	0	2.05^{bc} ±0.32	0.10^{c} ± 0.06	0^{b} ± 0	0.10^{a} ± 0.06
0	0	5	2.70^{a} ±0.21	0.25^{bc} ± 0.09	0.02^{b} ± 0.01	0.45^{bc} ± 0.18
1	0	5	1.61^{c} ± 0.18	0.20^{bc} ± 0.11	0.02^{b} ± 0.01	0.20^{b} ± 0.11
2	0	5	$2.30c^{ab}$ ± 0.12	2.70^{a} ± 0.43	0.86^{a} ± 0.24	1.35^{a} ± 0.20
3	0	5	2.51^{ab} ± 0.22	0.85^{b} ± 0.19	0.89^{a} ± 0.26	1.0^{ab} ± 0.22
2	0	0	0.83^{d} ± 0.02	0.70^{bc} ± 0.17	1.12^{a} ± 0.35	1.4^{a} ± 0.33
2	1	0	0.75^{d} ± 00	0.65^{bc} ± 0.15	0.85^{a} ± 0.22	0.65^{bc} ± 0.18
2	2	0	0.77^{d} ± 0.04	0.80^{b} ± 0.21	0.52^{ab} ± 0.16	1.05^{ab} ± 0.22
2	3	0	0.91^{d} ± 0.02	0.55^{bc} ± 0.11	0.05^{b} ± 0.01	0.55^{bc} ± 0.11

*Data represented as mean value ± standard error; different letters in the column are significantly different (Duncan Multiple Range Test $p\leq0.05$)

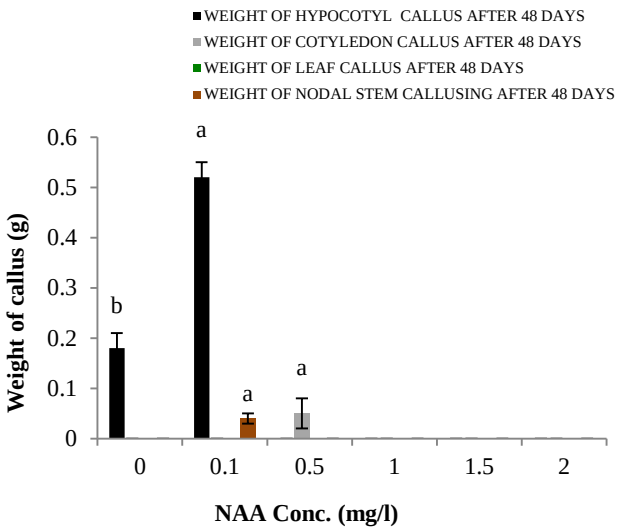

Fig. 1. Effect of different concentrations of NAA on weight of callus after 48 days of culturing.

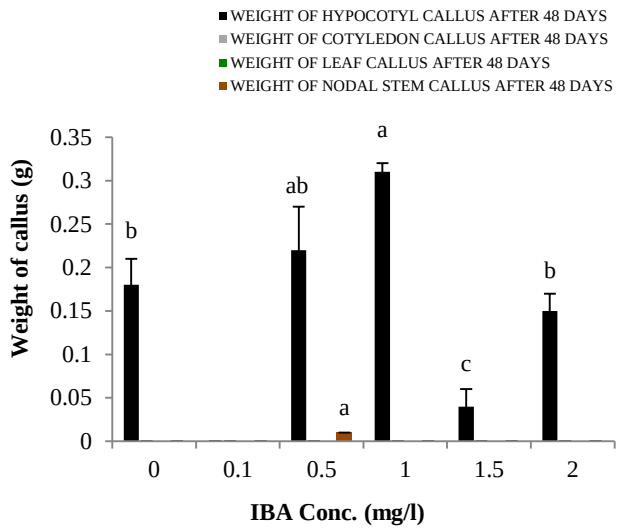

Fig. 2. Effect of different concentrations of IBA on callus weight after 48 days of culturing.

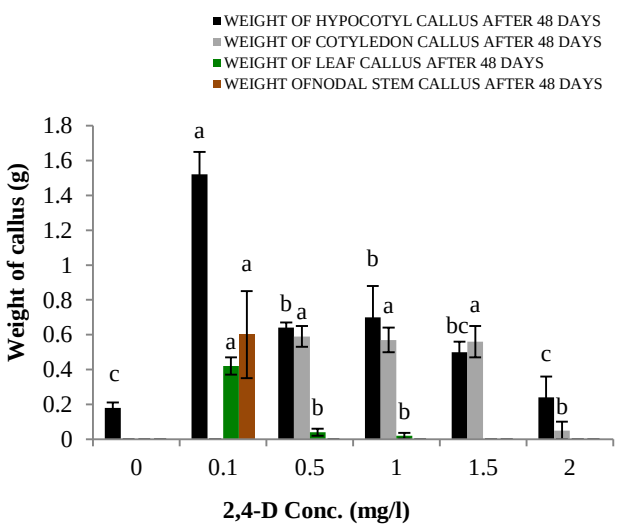

Fig. 3a. Effect of different concentrations of 2,4-D on callus weight after 48 days after culturing.

Fig. 3b. Callus derived from hypocotyl explant of *Brassica napus* L. in MS media containing 0.1 mg/l 2,4-D.

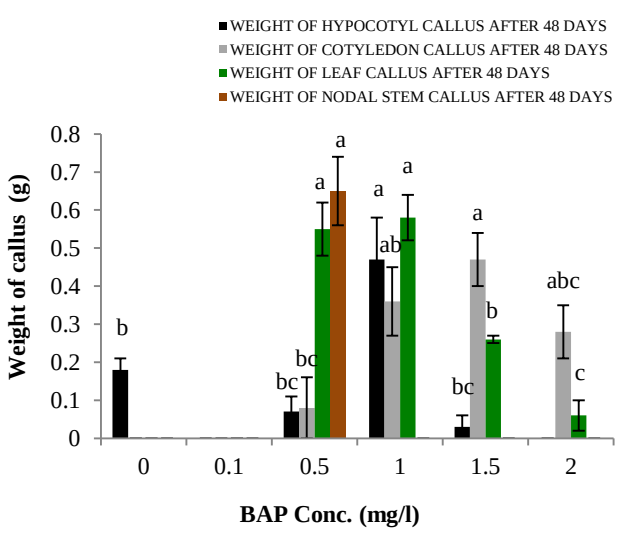

Fig. 4. Effect of different concentration of BAP on callus weight after 48 days of culturing.

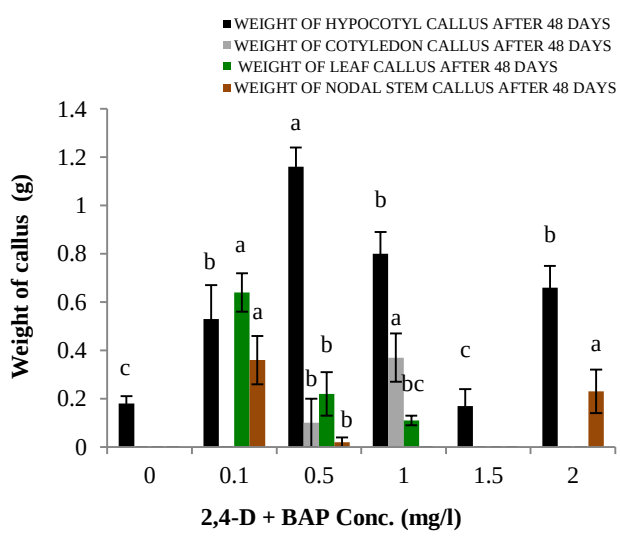

Fig. 5. Effect of equal concentrations of 2,4-D + BAP on callus weight after 48 days of culturing.

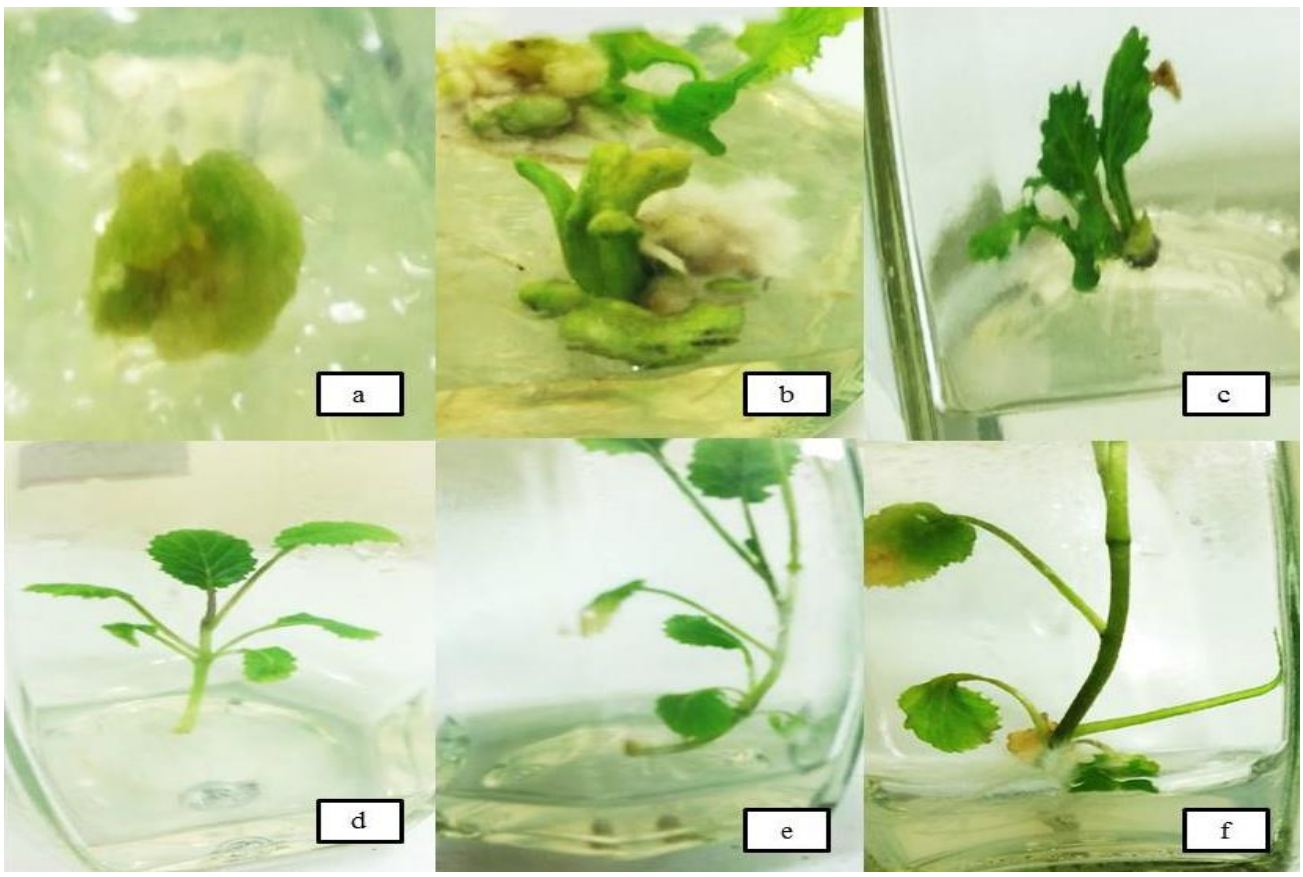

Fig. 6. Regeneration of *Brassica napus* L.var. Hyola- 401 from callus: a) Shoots initiation from callus on MS media containing augmented with 5 mg/l AgNO3 along with 2mg/l BAP. b) Shoot elongated on callus. c) Multiple shoots arising from hypocotyl derived calli on the same media. d) Rooting initiated after 10 days of inoculation on hormone free half MS media in *In vitro* regenerated shoots. e and f) Rooted plantlets after 30 days of inoculation on rooting media.

The combine treatment of 2,4-D and BAP also induced callus but less than 2,4-D alone (Fig. 5). In hypocotyl explant there was significant increase in callus growthwith increase in concentrations of combine treatment up to 0.5 mg/l further increase in the amount of growth regulators reduced the callus formation. Cotyledon explants was not much responsive by combined treatment. Callus induction in leaf explants and nodal stem was maximum at combine treatment of 2,4-D and BAP at 0.1 mg/l . Callus induction was reduced by the increase in concentrations of leaf explants. Similarly in nodal stem explant callus induction was inhibited with increase of concentrations but at 2mg/l (BAP+2,4-D) yellowish cream callus was induced.

Regeneration from calli: On shifting the calli to a hormone free medium after 4 weeks the colour of calli changed to yellow brown and few to green. Increased in weight of callus was observed and substantially a few shoots roused. $AgNO_3$ alone in MS media did not show any remarkable increase in the number of shootlets as compare to control, however an increase in callus weight was observed within 4 weeks. The effect of $AgNO_3$ along with BAP appeared to be more significant than $AgNO_3$ alone (Table 1). The most responsive medium for shoot regeneration was MS medium augmented with 5 mg/l $AgNO_3$ along with 2mg/l BAP (Fig. 6a) as the number of shoots formed was more as compare to other combinations of BAP (Table 1; Fig. 6b and 6c).

Culturing on BAP independently was found to increase the length of shoots as well as number of leaves, whereas the number of shoot and amount of callus formed was less as compared to other combinations. The combination of BAP with Kinetin in MS medium was found less responsive as compared to incorporation of BAP with $AgNO_3$ in MS medium.

Regenerated shoots were shifted to half strength MS media with various concentrations of IBA and hormone free media. From the all trials used the best rooting media was half strength (hormone free) media. When elongated regenerated shoots were shifted to half strength MS rooting media roots induced in all regenerated plantlets after 10-12 days (Fig. 6d). 6-10 roots per explant were observed with an average length of 7.98 cm (Fig. 6e and f).

Discussion

The results of present study revealed that hypocotyl was the best explant for callus induction in MS medium containing 0.1 mg/l 2,4-D as compared to other growth regulators like NAA, BAP, IBA or even the combination of 2,4-D with BAP. This result supports Ali *et al*., (2007), where the highest callus was obtained at 0.5mg/l of 2,4-D. Whereas Borjian and Arak (2013) reported interaction between BAP, NAA and 2,4-D hormones were more effective than 2,4-D alone in *Brassica napus* L. (SLM-046 var.).

Dubey & Gupta (2014) reported the best callus induction on MS media containing 0.5 mg/l 2,4-D with 0.5 mg/l NAA. Sharma and Gupta (2012) also used the higher amount of 2,4-D (1.5 mg/l)as compare to the present work for callus induction from *Brassica napus* L. hypocotyl segments on B5 medium. Alam *et al*., (2013) reported callus induction in *Brassica napus* L. hypocotyl and cotyledon on medium supplemented with 0.5 mg/l 2,4-D, 0.5 mg/l BAP and o.5 mg/l $AgNO_3$ and better callus production in hypocotyls than cotyledons. Similarly these finding are also not in consistent with Munir*et al*., (2008) who observed best callus induction in MS medium containing 0.5 mg/l IAA, 1 mg/l BAP,0.5 mg/l NAA and 1 mg/l Kinetin in *Brassica napus* hypocotyls. Neha & Ashtutosh (2014) reported 100% callus production in *Brassica juncea* on MS medium supplemented with BAP at 2-2.5 mg/l and NAA 0.5 mg/l. Jacobsen (1983), Naz & Khalida (2014) reported that higher efficiency of 2,4-D depends on its greater availability in tissues. Accumulation of 2,4-D in tissue occurs because of its higher mobility and of its restricted oxidation and conjugation rate as compare to other hormones like NAA having higher degradation rate and lower mobility justifying higher efficiency of 2,4-D.

Cytokinins play a vital role in regulating growth and morphogenesis in plants. Its main function is to induce shoots (Leopold, 1987). For the direct or indirect regeneration of *Brassica napus* L. BAP has been widely used (Burbulis *et al*., 2009).

In the present experiment the regenerative capacity of *Brassica napus* L. into plantlets via hypocotyl derived callus was estimated by trying nine different combinations of phytohormones in MS media. Shoot regeneration was observed at all concentrations of BAP. The maximum number of shoot regeneration was noticed in the medium fortified with 2mg/l BAP and 5mg/l $AgNO_3$.

Present results are in conformity with Burbulis *et al*., (2008) who reported maximum number of adventitious buds from hypocotyl derived callus of *Brassica napus* L. on MS medium supplemented with 4mg/l BAP and 0.05 mg/l NAA. Hussain *et al*., (2014) investigated shoot regeneration through hypocotyl derived callus in MS medium containing 5 mg/l BAP and 0.5 mg/l IAA.

Shoot regeneration was also reported in other species of *Brassica.* Abrha *et al*., 2014 examined successful shoot regeneration from callus on MS medium augmented with 2mg/l BAP in two verities of *Brassica carinata* i.e. yellow Dodola and Holeta-1.

Lone *et al*., 2017 was successful in regenerating *Brassica juncea* through hypocotyl derived callus on MS medium supplemented with 0.5 mg/l 2,4-D and 5mg/l BAP in variety RSPR 03 and on MS medium supplemented with 0.5 mg/l 2,4-D and 4mg/l BAP in cultivar RSPR 01.This is not in accordance with Munir *et al*., (2008) who obtained best results when callus was cultured on MS medium containing 1mg/l Zeatin and 0.1mg/l IAA in *Brassica napus* L. Incorporation of Adjuvants $AgNO_3$ to MS regeneration medium used was significantly effective to *Brassica napus* L. Its stimulating effect on shoot regeneration wasreported by different workers.

Sharma & Gupta (2012) reported maximum shoot regeneration on MS medium containing 3mg/l Kinetin, 0.5 mg/l NAA and 1mg/l $AgNO_3$. Ali *et al*., (2007) demonstrated shoot regeneration from callus using NAA, 2mg/l BAP and 5mg/l $AgNO_3$ in MS medium. Alam *et al*., (2013) reported best shooting media for *Brassica napus* L. was 3mg/l BAP, 0.1mg/l NAA and 5 mg/l $AgNO_3$.

For inducing roots different trial was used and the best result rooting media used was half MS media (hormone free). These results are in conformity with earlier reports. Sharma & Gupta (2012) observed maximum number of roots from a media fortified with half strength. These results are not in conformity with Alam *et al*., (2013) who obtained best root system in MS media supplemented with 2.5 mg/l NAA and 1 mg/l IBA. Hussain *et al*., (2014) used MS media containing 0.125 mg/l IAA and 0.250 mg/l IBA for rooting of *In vitro* regeneration shoots. Burbulis *et al*., (2008) achieved rooting of *In vitro Brassicanapus* L. on MS media augmented with 0.1 mg/l NAA. Ali *et al*., (2007) used half strength media supplemented with 0.3 mg/l IBA in 3cultivars of *Brassica napus* L. (star, Westar and cyclone). Chamandosti *et al*., (2006) observed rooting on MS media containing 1mg/l IBA in Canola.

In further research this standardized callus induction protocol might be used for regeneration of salt tolerant *Brassica napus* L. via indirect regeneration method.

Conclusion

On the basis of present study it may be concluded that the efficiency of callus induction in hypocotyl explant was best achieved in MS medium containing 0.1 mg/l 2,4-D and regeneration of plantlet through callus was best achieved in MS medium containing 2mg/l BAP and 5mg/l $AgNO_3$. Hence by using these protocols, the extensive use of high amount of growth regulators could be reduced for *In vitro* propagation of elite cultivars of *Brassica napus* L.

References

Abrha, G.T., F. Mekbib and B. Amassu. 2014. *In vitro* propagation of Ethopian mustard (*Brassica crianta* A. Braun). *Afr. J. Biotechnol*., 13(4): 4438-4448.

Alam, S.S., L. Khaleda and M. Al-Forkan. 2013. Establishment of regeneration protocol for canola (*Brassica napus* L.). *GJSFR*., 13(7): 7-11.

Ali, H., Z. Ali, H. Ali, S. Mehmood and W. Ali. 2007. *In vitro* regeneration of *Brassica napus* L., cultivars (star, cyclone and westar) from hypocotyls and cotyledonary leaves. *Pak. J. Bot*., 39(4): 1251-1256.

Borjian, L. and H. Arak. 2013. A study on effect of different concentration of plant Hormones (BAP, NAA, 2,4- D and Kinetin) on callus induction in *Brassica napus*. *IRJABS*., 5(4): 519-521.

Burbulis, N., A. Blinstrubiene, R. Kupriene, V. Jaytiene, R. Rugienins and G. Staniene. 2009. *In vitro* regeneration of *Brassica napus* L. shoots from hypocotyls and stem segments. *Zemdirbyste–Agriculture*, 96(3): 176-185.

Burbulis, N., R. Kuprieneand A. Blinstrubiene. 2008. Callus induction and plant regeneration from somatic tissue in spring rapeseed (*Brassica napu* L.). *Biologija*, 54(4): 258-263.

Chamandosti, F., A. Majd and S.Mehrabian. 2006. *In vitro* plant regeneration from callus of cotyledons in canola (*Brassica napus* L.). *J. Biol. Sci*., 9(2): 302-306.

Dubey, S.K. and A.K. Gupta. 2014. Callus induction and shoot proliferation from seedling explants of different mustard genotypes. *Int. J. Curr. Microbiol. App. Sci*., 3(7): 858-864.

FAO statistics. 2018. www.fao.org/statistics.

Fay, M.F. 1992. Conservation of rare and endangered plants using *In vitro* methods. *In Vitro Cell Dev. Biol*., 28(1): 1-4.

Hassan, S., I. Khan and M.A. Javaid. 1994. Callus induction and regeneration of plants from anther of rapeseed. *Pak. J. Agri. Res*., 15(1): 32-36.

Hussain, S., A. Rasheed, M. Latif, T. Mahmood and S.M. Saqlannaqvi. 2014. Canola (*Brassica napus* L.) regeneration and transformation via hypocotyl and hypocotyl derived calli. *Sarhad J. Agric*., 30(2): 165-172.

Jacobsen, H.J. 1983. Biochemical mechanism of plant hormone. In: *Handbook of Plant Cell Culture*. (Eds.): Evans, D.A., W.R. Sharp, P.V. Ammirato and Y. Yamada, Mac Millan, pp. 672-695.

Jain, R.K., S. Jain, H.S. Ninawatee and J.B. Choudhury. 1990. Salt tolerance in *Brassicajuncea* L. *In vitro* selection, agronomic evaluation and genetic stability. *Euphytica*, 48(2): 141-152.

Katiyar, R.K. 1997. Exploitation of variability generated through somaclonal route in mustard (*Brassica juncea*). *Ann. Agil. Res*., 18(4): 512-517.

Kharb, P., J.B. Choudhry and R.K. Jain. 2002. Assessment of somaclonal variation in three tetreploid species of *Brassica*. *Nat. J. Pl. Improv*., 4(2): 30-34.

Khare, C.P. 2007. *Indian Medicinal plants*. Springer. Delhi, India.

Larkin, P.J. and W.R. Scowcroft. 1981. Somaclonal variation a novel source of variability from cell culture for plant improvement. *Theor. Appl. Genet*., 60: 197-214.

Leopold, A.C. 1987. Contemplation on hormones as biological regulators. In: Hormone action in plant development action. A critical appraisal. (Eds.) G.V. Hoad, J.R. Lenton, M.B. Jackson and R.K. Atkins, Butterworths, London, Bosen Durban, Singapore, Sydney, Toronto, Wellington, pp. 3-15.

Lin, L., H. Allemekinders, A. Dansby, L. Campbell, S. Durance-Tod, A. Berger and P.J.H. Jones. 2013.Evidence of health benefits of canola oil. *Nutrition Reviews*, 71(6): 370-385.

Lone, J.A., S.K. Gupta, S.H. Wani, M. Sharma, R.A. Lone and A.B. Shikari. 2017. Efficient callus induction and regeneration in *Brassica juncea* for environmental friendly Agriculture. *Int. J. Pure App. Biosci*., 5(1): 135-141.

Munir, M., H. Rashid, M. Rauf, Z. Chaudhry and M.S. Bukhari. 2008. Callus formation and plantlets regeneration from hypocotyls of *Brassica napus* by using different media combination. *Pak. J. Bot*., 40(1): 309-315.

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

Naz, S. and K. Khatoon. 2014. The effect of auxin on callus induction in *Achyranthesaspera*. *Pak. J. Bot*., 46(6): 2203-2207.

Neha, T. and D. Ashutosh. 2014. Efficient callus regeneration and multiple shoot induction in *Brassica juncea* var. *PusaJaikisan*. *Res. J. Recent Sci*., 3: 2277-2502.

Pakistan Bureau of Statistics. 2015. Ministry of food, Agriculture and Livestock, Islamabad, Pakistan.

Qamarunnisa, S., I. Jamil, S. Raza, A. Azhar and S.H.M. Naqvi. 2015. Genetic improvement of Canola against abiotic stress through incorporation of Dreb. *Asian J. Agri. Biol*., 3(3): 97-104.

Ray, A.B., J.P.N. Chansouria and S. Hemalatha. 2010. *Medicinal Plants: Antidiabetic and Hypoglycaemic activities*, 1 ed. ibdc, Lucknow, India.

Sharma, M. and S.K. Gupta. 2012. Effective callus induction and plant regeneration in *Brassica napus* (L.) var Dgs-1. 2012. *J. Cell & Tiss. Res*., 12(2): 3229-3234.

Vaughan, J.G. and C.A. Geissler. 2009. *The new oxford book of food plants*, Oxford University press, New York, United states.

(Received for publication 15 April 2017)