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Thidiazuron Induces High Frequency Shoot Regeneration in Leaf and Petiole Explants of Cabbage (*Brassica Oleracea L. Var. Capitata*)

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Abstract

We have studied the effect of thidiazuron on shoot regeneration from leaf and petiole explants of cabbage (*Brassica oleracea* L. var. *capitata* cv. Pride of India). Different concentrations and combinations of thidiazuron (alone), thidiazuron+ Adenine, thidiazuron+ NAA and thidiazuron+ IAA were used in MS medium to optimize *in vitro* shoot regeneration from leaf and petiole explants of cabbage (*Brassica Oleracea* L. var. *capitata* cv. Pride of India). Leaf and petiole explants procured from 20-25 days old glasshouse grown seedlings of cabbage. Out of 40 combinations and concentrations tried separately for leaf and petiole explants, the high frequency shoot regeneration from leaf (91.11%) and petiole (88.88%) explant was obtained on MS medium supplemented with0.220mg/l thidiazuron + 0.02 mg/l NAA and 0.330 mg/l thidiazuron + 0.02 mg/l NAA respectively. High percentage root regeneration (100%) in *in vitro* developed shoots was obtained on MS medium supplemented with 0.10mg/l NAA. The regenerated complete plantlets were transferred to pots containing sterilized coco peat and acclimatized. A high efficiency and reliable plant regeneration protocol has been developed using thidiazuron. This regenerated protocol would be useful for genetic transformation studies in cabbage.

Keywords: Acclimatization; *In vitro* regeneration; Leaf; Petiole; Cabbage; Thidiazuron

Abbreviations: NAA-Naphthalene acetic acid; IAA- Indole-3-acetic acid; MS- Murashige and Skoog

Introduction

Cabbage (*Brassica Oleracea* L. var. capitata) is an important vegetable crop of the family Brassicaceae and is used as a leafy green vegetable. The Food and Agriculture Organization of the United Nations (FAO) reports that world production of cabbage and other brassica's calendar year in the 2011 was 68,840,531 metric tons and cultivated on 2,373,818 ha land. This was primarily grown in China (43 percent) and India (11 percent) with production of 79,49,000 metric tons on 369,000 ha land). It is excellent source of vitamin C and also contains significant amount of glutamine, an amino acid that has anti-inflammatory properties. Cabbage contains glucosinolates and sulphoraphane which serve as metabolic detoxicants and has anticarcinogenic activities respectively. It is also a source of indole-3-carbinol, a chemical which boosts DNA repair in cells and appears to block the growth of cancer cells [1].

In vitroregeneration offers a great opportunity for a rapid production of desirable and essentially genetically identical plants [2]. An efficient *in vitro* regeneration system is also a crucial tool in genetic engineering of the crop for improved characteristics [3]. Most transformation protocols use a regeneration system which start with physical isolation of the explants followed by exposure to a suitable plant growth regulators regime to activate developmental pathway, either somatic embryogenesis or organogenesis. In many crops, this strategy successfully accomplished by optimizing the type of explants, growth regulators and the nutritional and physical conditions for its culture [4].

In the present investigation, the effects of thidiazuron on shoot regeneration from leaf and petiole explants of cabbage are described. Over time, a large number of reports underlined the high frequency shoot regeneration from various explants of cabbage (*Brassica oleracea* L. var. *capitata*). Plant regeneration studies have been carried in cabbage using various explants such as cotyledons [5-15], hypocotyl [5,6,8,12,14-19], leaf [20-24], petiole [3,10,25], protoplast [26-28] and another culture [29,30]. In this paper, we report a simple, high frequency direct and indirect shoot regeneration procedure using different concentration of thidiazuron alone and in combinations with Adenine, NAA and IAA from leaf and petiole explants of cabbage (*Brassica oleracea* L. var. *capitata* cv. Pride of India). We are currently using this plant regeneration system for the genetic transformation of cabbage by genetically engineered *Agrobacterium tumefaciens* strain containing *cry IAa* gene

Material and Methods

Plant material and culture medium

The certified seeds of cabbage (*Brassica oleracea* var. *capitata* cv. Pride of India) were procured from the Department of vegetable crop, Dr Y.S. Parmar University of Horticulture and Forestry. The seeds of cabbage were soaked for an hour in water and then sown in pots. 20-25 days old glass house grown seedlings were used as a source of explants i.e. leaf and petiole, respectively, to carry out plant regeneration and genetic transformation studies in cabbage. The explants (leaf and petiole) were surface and cultured on MS salt (macro and micro), vitamins supplemented with 100 mg/l meso-inositol, 3 percent sucrose and 0.8 percent agar agar was also used as basal medium in shoot regeneration experiments. Different concentrations and combinations of cytokinins and auxins [thidiazuron alone, thidiazuron+Adenine, thidiazuron+NAA, thidiazuron+IAA] were used in the MS basal medium. The pH of the medium was adjusted to 5.8 before adding agar

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agar. The medium was poured in culture vessels and sterilized at 1.08 kg/cm^{2}for 15 minutes in an autoclave. All the aseptic manipulations were carried out under laminar air flow chamber.

Plant regeneration from leaf and petiole explants

For obtaining high efficiency shoot regeneration, leaf and petiole explants were excised from glass house grown 20-25 days old seedlings and surface sterilized. The explants were cut into small pieces and their surface was gently tapped with the scalpel blade to injure them. These explants were cultured in flasks containing full strength MS basal medium supplemented with various combinations and concentrations of plant growth regulators such as thidiazuron (mg/l), thidiazuron+Adenine (mg/l), thidiazuron+NAA (mg/l) and thidiazuron+IAA (mg/l) (All the plant growth regulators have been filter sterilized). For every combination 5 flasks with 5 explants were inoculated and each experiment was repeated thrice. Observations were taken at the interval of 7 days till shoot regeneration. Explants were evaluated for average number of shoots per explants and percentage shoot regeneration. After inoculation, the culture vessels were kept in the culture room at 26 ± 2°C under 16 hrs photoperiod with cool fluorescent lamps (40 mmol/m^2/s) having 70-80% humidity. The regenerated shoots (2-3 cm), obtained from both the explants were separated and individual shoot was transferred to the MS medium containing various concentrations of different auxins IAA and NAA for root induction to get a complete plantlets. These were then evaluated for percentage of root regeneration after 4 weeks of culturing (Figure 1).

Hardening of regenerated plantlets

After proper *in vitro* development, the regenerated plantlets were taken out of the tubes in such a way that no damage was caused to their root system. The roots were washed gently under running tap water to remove adhering medium. After removal of the medium, plantlets were kept in running tap water for a few minutes so that they do not wilt after transfer to soil. The plantlets were treated with 0.5 % bavistin for 2-3 minutes. The *in vitro* regenerated plantlets after washing were transferred to the presterilized coco peat mixture. After transfer of the plantlets to presterilized cocopeat mixture they were watered with 0.5 % bavistin solution and covered with polythene bags to maintain relative humidity. These were transferred to the culture room in which temperature and light conditions were controlled. Water was sprayed twice a day to maintain relative humidity. After a week, when plants showed initial signs of establishment in pots with appearance of new leaves, the polythene bags were removed. The plantlets were transferred to pots containing presterilized potting mixture (consisting of sand + soil + FYM). The potted plantlets were kept under glass house. The percentage survival of the hardened plants was recorded 5 weeks after transfer.

Data analysis

Each treatment consisted of at least 25 explants and each experiment was repeated thrice. The data recorded for the different parameters were subjected to Completely Randomized Design. The statistical analysis based on mean values per treatment was made using analysis of variance for Completely Randomized Design (CRD).

Results

Shoot regeneration from leaf and petiole explants

Young and tender leaf and petiole explants were excised from the plants raised in pots in glasshouse. After surface sterilization, both explants were cut into small segments of 0.5–1.0 cm size and

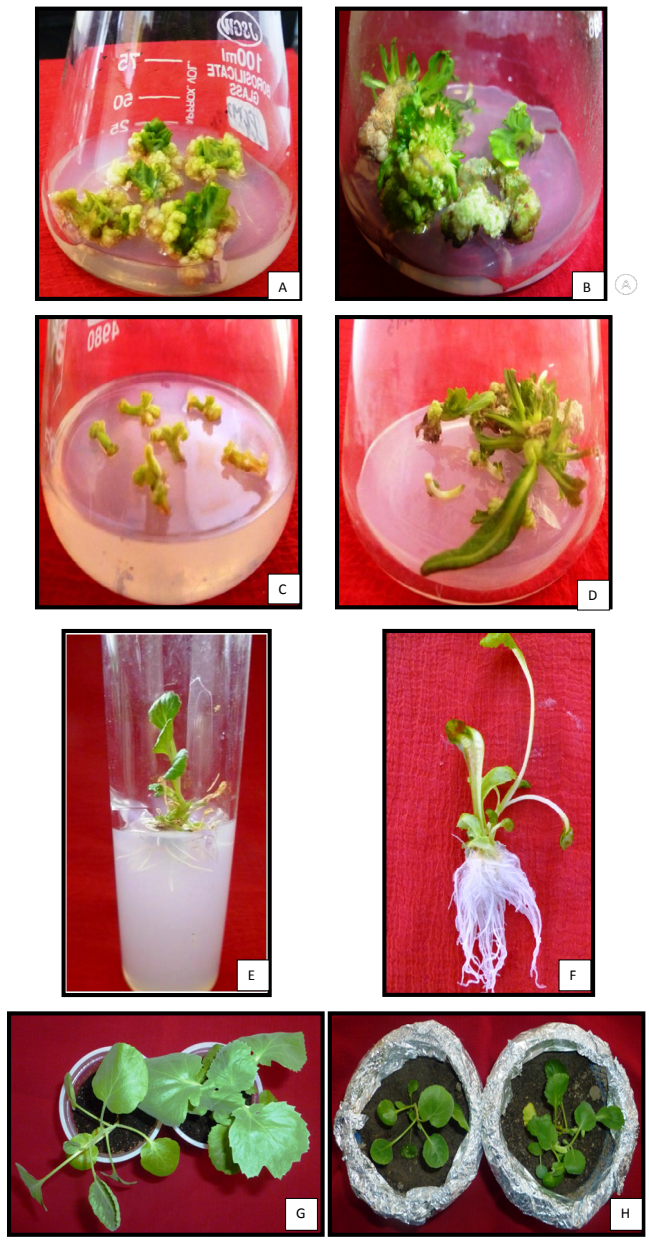

Figure 1: Plant regeneration from leaf and petiole explants of cabbage (Brassica oleracea L. var. capitata cv. Pride of India).

a. Callus formation obtained from leaf explants on medium supplemented with (MS+ 0.22mg/l TDZ + 0.02mg/l NAA).
b. Shoot regeneration from leaf explants on medium supplemented with (MS+ 0.22mg/l TDZ + 0.02mg/l NAA).
c. Callus formation obtained from petiole explants on medium supplemented with (MS+ 0.22mg/l TDZ + 0.02mg/l NAA).
d. Shoot regeneration from petiole explants on medium supplemented with (MS+ 0.22mg/l TDZ + 0.02mg/l NAA).
e. Root regeneration from the *in vitro* developed shoot regeneration from explants on the medium supplemented with (MS + 0.10mg/l NAA).
f. Fully developed plantlets of cabbage taken out of medium (after proper washing) after 15 days of culturing showing well developed root system. (Later on transferred to pots containing pre-sterilized cocopeat for acclimalization).
g. Regenerated plantlets transferred to plastic pots containing mixture of pre-sterilized cocopeat for hardening.
h. Plantlets transferred to potting mixture (containing sand + soil + FYM).

cultured on MS medium supplemented with various concentrations and combinations of thidiazuron (mg/l), thidiazuron+Adenine (mg/l), thidiazuron+NAA (mg/l) and thidiazuron+IAA (mg/l). The colour of both explants did not change. No change in colour of media was observed.

Effect of thidiazuron (in MS medium) on shoot regeneration: The leaf and petiole explants were inoculated on MS medium supplemented with ten different concentrations of thidiazuron for shoot regeneration. Swelling and expansion in the both explants were observed at the initial days of culture. No callusing was recorded in leaf explants except three concentrations (E4, E5 and E10) whereas, callusing was observed in petiole explants after 18-22 days at lower concentrations of thidiazuron whereas 33-38 days at higher concentrations of thidiazuron. Shoot regeneration in leaf explants was observed after 72-80 days of culturing was recorded at all concentrations of thidiazuron. In petiole explants after 35-40 days at lower concentrations of thidiazuron and 72-75 days at higher concentrations after culturing whereas, The maximum percent shoot regeneration (80.00%) and (84.44%) average number of shoots per explant (1.95)and (2.660) was observed on E5 medium (MS basal + 0.550mg/l thidiazuron). The regenerated shoots were transferred to the same medium for multiplication and elongation (Table 1).

Effect of thidiazuron and Adenine (in MS medium) on adventitious shoot bud regeneration: The leaf and petiole explants were inoculated on MS medium supplemented with ten different combinations and concentrations of thidiazuron and Adenine for shoot regeneration. Swelling and expansion in both explants was observed at the initial days of culture. Direct shoot regeneration in leaf explants was observed in seven concentrations whereas three concentrations (F5, F6 and F10) showed callusing after 27-32 days of culture and in petiole explants the callus was observed after 15-17 days of culture in all concentrations. Shoot regeneration was observed after 62-65 and 35-40 days of culturing in leaf and petiole explants respectively. The maximum percent shoot regeneration (68.88%) and (85.18%) and maximum average number of shoots per explants (2.107) and (2.90) was observed on F2 medium (MS basal + 0.220mg/l thidiazuron + 79.7mg/l adenine) and F7 medium (MS basal + 0.770mg/l thidiazuron + 79.7mg/l adenine). The regenerated shoots were transferred to the same medium for multiplication and elongation (Table 2).

Effect of thidiazuron and NAA (in MS medium) on shoot bud regeneration: The leaf and petiole explants were inoculated on MS medium supplemented with ten different combinations and concentrations of thidiazuron and NAA for shoot regeneration.

Medium code	Medium Composition	Average number of shoots regenerated per explants	Percent shoot regeneration	Average number of shoots regenerated per explants	Percent shoot regeneration
		LEAF		PETIOLE	
E1	MS basal medium + 0.110mg/l TDZ	1.197	46.66(43.08)	0.606	27.77(31.73)
E2	**MS basal medium + 0.220mg/l TDZ**	**0.773**	**24.44(29.58)**	1.163	33.33(35.21)
E3	MS basal medium + 0.330mg/l TDZ	1.420	51.11(45.64)	0.736	25.92(30.58)
E4	MS basal medium + 0.440mg/l TDZ	1.377	53.33(46.91)	1.363	55.55(48.20)
E5	**MS basal medium + 0.550mg/l TDZ**	**1.953**	**84.44(66.87)**	**2.660**	**80.00(63.64)**
E6	MS basal medium + 0.660mg/l TDZ	1.400	62.22(52.09)	0.716	22.22(28.01)
E7	MS basal medium + 0.770mg/l TDZ	1.287	64.44(53.41)	0.689	24.07(29.35)
E8	MS basal medium + 0.880mg/l TDZ	1.087	42.22(40.52)	0.553	24.07(29.35)
E9	MS basal medium + 0.990mg/l TDZ	1.130	46.44(43.08)	**0.00**	**0.00(0.00)**
E10	MS basal medium + 1.100mg/l TDZ	0.997	44.44(41.80)	1.253	48.15(43.94)

*The values in parenthesis are arc sine transformed values.

$CD_{0.05}$ 0.2281 7.470(4.517) 0.2252 6.908(4.386)

SE± 0.1093 3.581(2.165)0.1079 3.312(2.102)

Table 1: Effect of various concentrations of TDZ alone (in MS medium) on shoot regeneration from leaf and petiole explants of cabbage (*Brassica oleracea* L.var. *capitata* cv. Pride of India).

Medium Code	Medium Composition	Average number of shoots regenerated per explants	Percent shoot regeneration	Average number of shoots regenerated per explants	Percent shoot regeneration
		LEAF		PETIOLE	
F1	MS basal medium + 0.110mg/l TDZ + 79.7 mg/l adenine	1.663	66.66(54.80)	2.830	77.77(61.99)
F2	**MS basal medium + 0.220mg/l TDZ + 79.7 mg/l adenine**	**0.640**	**24.44(29.58)**	**2.900**	**85.18(67.44)**
F3	MS basal medium + 0.330 mg/l TDZ + 79.7mg/l adenine	1.397	55.55(48.20)	2.517	83.33(66.10)
F4	MS basal medium + 0.440 mg/l TDZ + 79.7mg/l adenine	0.820	46.66(43.08)	1.200	37.03(37.47)
F5	MS basal medium + 0.550 mg/l TDZ + 79.7mg/l adenine	1.240	46.66(43.08)	1.423	53.70(47.13)
F6	MS basal medium + 0.660 mg/l TDZ + 79.7mg/l adenine	0.620	35.55(36.59)	2.400	71.37(57.69)
F7	**MS basal medium + 0.770 mg/l TDZ + 79.7mg/l adenine**	**2.107**	**68.88(56.12)**	1.310	37.03(37.47)
F8	MS basal medium + 0.880 mg/l TDZ + 79.7mg/l adenine	1.240	44.44(41.80)	1.383	44.44(41.80)
F9	MS basal medium + 0.990 mg/l TDZ + 79.7mg/l adenine	1.197	44.44(41.80)	**0.903**	**35.18(36.37)**
F10	MS basal medium + 1.10 mg/l TDZ + 79.7mg/l adenine	1.063	40.00(39.19)	1.753	70.37(57.04)

*The values in parenthesis are arc sine transformed values.

$CD_{0.05}$0.2120 8.793(5.224) 0.1818 7.086(4.723)

SE±0.1016 4.215(2.504) 0.0871 3.397(2.264)

Table 2: Effect of various concentrations and combinations of TDZ and Adenine (in MS medium) on shoot regeneration from leaf and petiole explants of cabbage (*Brassica oleracea* L. var. *capitata* cv. Pride of India).

Swelling and expansion in both explants was observed at the initial days of culture. The callus initiation was observed after 19-24 and 18-25 days after culturing in leaf and petiole explants. Shoot regeneration was observed after 45-55 and 35-50 days of culturing. The maximum percent shoot regeneration (91.11%) and (88.88%) and maximum average number of shoots (3.202) and (3.44) was observed on G2 medium (MS basal + 0.220mg/l thidiazuron + 0.02mg/l NAA) and G3 medium (MS basal + 0.330mg/l thidiazuron + 0.02mg/l NAA). The regenerated shoots were transferred to the same medium for multiplication and elongation (Table 3).

Effect of thidiazuron and IAA (in MS medium) on shoot regeneration: The leaf and petiole explants were inoculated on MS medium supplemented with ten different combinations and concentrations of thidiazuron and IAA for shoot regeneration. Swelling and expansion in both explants were observed at the initial days of culture. In both explants, the callus initiation was observed after 18-24 days at higher concentrations of thidiazuron whereas lower concentrations of thidiazuron showed direct shoot regeneration. Shoot regeneration was observed after 45-60 and 35-45 days of culturing in leaf and petiole explants respectively. The maximum percent shoot regeneration (33.33%) and (85.18%) in H6 and H2 medium. Maximum average number of shoots (0.73) and (2.72) was observed on H9 medium (MS basal + 0.990mg/l thidiazuron + 0.088mg/l IAA) and H2 medium (MS basal + 0.220mg/l thidiazuron + 0.088mg/l IAA). No shoot regeneration was observed in media H3, H4 and H5 for leaf explants. The regenerated shoots were transferred to the same medium for multiplication and elongation (Table 4).

Root regeneration from *in vitro* developed shoots

The *in vitro* regenerated shoots were excised from explants/callus and cultured on MS medium containing different concentration of auxins. Root regeneration was observed after 9-12 days of inoculation on medium supplemented with IAA and 7-9 days in NAA. Maximum percent root regeneration was observed 97.20% and 100% in IAA and NAA respectively. After 18-25 days complete root system has been developed. Callusing was observed in IAA whereas no callusing was observed on NAA (Table 5).

Hardening of regenerated plantlets of cabbage

After the complete development of roots i.e. about 20-25 days of rooting, cabbage plantlets were taken out of the culture tubes, taking various precautions to avoid any damage to the delicate root system. The roots were washed gently under running tap water to remove adhering medium and then plants were kept in 0.5 per cent bavistin solution for 5 – 10 minutes. Plastic cups were first filled to more than

Medium Code	Medium Composition	Average number of shoots regenerated per explants	Percent shoot regeneration	Average number of shoots regenerated per explants	Percent shoot regeneration
		LEAF		PETIOLE	
G1	**MS basal medium + 0.110 mg/l TDZ + 0.02 mg/l NAA**	**0.930**	**31.11(33.87)**	1.180	38.88(38.58)
G2	**MS basal medium + 0.220 mg/l TDZ + 0.02 mg/l NAA**	**3.020**	**91.11(72.88)**	2.310	81.48(64.56)
G3	MS basal medium + 0.330mg/l TDZ + 0.02 mg/l NAA	1.910	62.22(52.13)	**3.440**	**88.88(70.93)**
G4	MS basal medium + 0.440mg/l TDZ + 0.02 mg/l NAA	2.950	88.88(70.73)	1.053	36.85(37.36)
G5	MS basal medium + 0.550mg/l TDZ + 0.02 mg/l NAA	2.620	80.00(63.64)	1.403	40.73(39.65)
G6	MS basal medium + 0.660mg/l TDZ + 0.02 mg/l NAA	2.220	71.11(57.52)	1.253	48.15(43.94)
G7	MS basal medium + 0.770mg/l TDZ + 0.02 mg/l NAA	1.530	51.11(45.64)	1.053	37.03(37.47)
G8	MS basal medium + 0.880mg/l TDZ + 0.02 mg/l NAA	2.620	84.44(67.51)	0.923	35.18(36.37)
G9	MS basal medium + 0.990mg/l TDZ + 0.02 mg/l NAA	2.350	86.66(69.02)	**0.660**	**22.22(28.01)**
G10	MS basal medium + 1.10 mg/l TDZ + 0.02 mg/l NAA	2.443	82.22(65.15)	2.033	62.96(52.55)

*The values in parenthesis are arc sine transformed values.
$CD_{0.05}$ 0.18699.941(7.551)0.16166.890(4.804)
SE±0.08964.765(3.620)0.07753.303(2.303)

Table 3: Effect of various concentrations and combinations of TDZ and NAA (in MS medium) on shoot regeneration from leaf and petiole explants of cabbage (*Brassica oleracea* L. var. *capitata*cv. Pride of India).

Medium Code	Medium Composition	Average number of shoots regenerated per explants	Percent shoot regeneration	Average number of shoots regenerated per explants	Percent shoot regeneration
		LEAF		PETIOLE	
H1	MS basal medium + 0.110 mg/l TDZ + 0.088 mg/l IAA	0.710	26.66(31.09)	1.627	57.40(49.27)
H2	MS basal medium + 0.220 mg/l TDZ + 0.088 mg/l IAA	0.573	22.22(28.07)	2.033	75.92(60.65)
H3	MS basal medium + 0.330mg/l TDZ + 0.088 mg/l IAA	0.000	0.00(0.00)	1.180	38.88(38.55)
H4	MS basal medium + 0.440mg/l TDZ + 0.088 mg/l IAA	0.000	0.00(0.00)	**2.720**	**85.18(67.44)**
H5	MS basal medium + 0.550mg/l TDZ + 0.088 mg/l IAA	0.000	0.00(0.00)	1.770	70.37(57.04)
H6	**MS basal medium + 0.660mg/l TDZ + 0.088 mg/l IAA**	**0.686**	**33.33(35.26)**	1.643	61.11(51.42)
H7	MS basal medium + 0.770mg/l TDZ + 0.088 mg/l IAA	0.573	24.44(29.58)	0.923	37.03(37.47)
H8	MS basal medium + 0.880mg/l TDZ + 0.088 mg/l IAA	0.420	20.00(26.57)	**0.736**	**31.48(34.11)**
H9	MS basal medium + 0.990mg/l TDZ + 0.088 mg/l IAA	0.730	31.11(33.87)	0.957	37.03(37.47)
H10	MS basal medium + 1.10 mg/l TDZ + 0.088 mg/l IAA	0.886	31.11(33.87)	1.553	51.85(46.06)

*The values in parenthesis are arc sine transformed values.
$CD_{0.05}$ 0.1491 4.145(2.706)0.18335.728(3.592)SE±0.07151.987(1.297)0.08792.746(1.722)

Table 4: Effect of various concentrations and combinations of TDZ and IAA (in MS medium) on shoot regeneration from leaf and petiole explants of cabbage (*Brassica oleracea* L. var. *capitata* cv. Pride of India).

S.No.	Medium Composition	IAA	NAA
1.	MS basal medium + 0.05 mg/l	97.20(84.39)	77.78(61.97)
2.	**MS basal medium + 0.10 mg/l**	97.20(84.39)	**100(90.00)**
3.	MS basal medium + 0.20 mg/l	86.09(68.32)	94.40(78.77)
$CD_{0.05}$ 7.796(10.628)			
SE± 3.711(5.058)			

*The values in parenthesis are arc sine transformed values.

Table 5: Effect of different concentrations of IAA and NAA on per cent root regeneration from *in vitro* developed shoots of cabbage (*Brassica oleracea* L. var. *capitata* cv. Pride of India).

half of their capacity with pre-sterilized cocopeat. The root portion of the plantlets were then placed over the cocopeat and covered with sterilized cocopeat gently to full capacity of the cup. The plantlets were watered and covered with polythene bags to maintain high humidity. Water was sprayed daily. After 13-15 days, when plants show initial sign of establishment in plastic pots with appearance of new leaves, they were uncovered overnight and after 21 days were fully uncovered. After keeping, them in these cups containing cocopeat they were transferred to the pots containing potting mixture (Sand: soil: FYM mixture). The plants were watered adequately daily. Maximum percent survival was 80% percent.

Discussion

In the present investigation, TDZ (thidiazuron) was used in MS medium for shoot regeneration studies. thidiazuron is a synthetic phenylurea cytokinin like compound that has been proven to be highly effective regulator of shoot morphogenesis [31-33]. It is also effective in terms of shoot regeneration in many recalcitrant species [34-37]**.** The effect of various concentrations of thidiazuron alone and in combination with Adenine, NAA and IAA were studied for enhancing the shoot regeneration frequency from leaf and petiole explants in cabbage (*Brassica oleracea* L. var. *capitata* cv. Pride of India). Different explants responded with different intensity on MS medium supplemented with thidiazuron in combination with Adenine and different auxins. Many published protocols for *Brassica* species regeneration whether they include transformation step or not are based on thidiazuron [38-44] and all the workers reported that the thidiazuron based media found to be very efficient for enhancing the frequency of shoot regeneration in Brassicaspecies.

During the present study, thidiazuron was found to be superior over the various cytokinin BAP and Kinetin (data not shown) in promoting shoot regeneration from leaf and petiole explants in cabbage. In present studies, the maximum percent shoot regeneration from leaf explants (91.11%) was obtained on thidiazuron + NAA supplemented medium. Similar result was observed by Ravanfar et al. 2014a in cabbage (cotyledon explant). In the present investigation, out of 40 combinations and concentration of thidiazuron (alone), thidiazuron+Adenine, thidiazuron+NAA and thidiazuron+IAA tried for both the explants. Only three medium (thidiazuron+IAA) for leaf and one medium (thidiazuron alone) for petiole were ineffective (Table 4 and 5). thidiazuron had induced high frequency shoot regeneration leaf and petiole explants. Whereas, Cheng et al. (2001) reported that hypocotyl explants showed better result on MS medium supplemented with thidiazuron as compare to MS medium supplemented with BA. Lu *et al.* (2003) have also reported that thidiazuron was more effective than BA. In the medium supplemented with thidiazuron, it was more efficient and rapid for shoot regeneration and regeneration frequency reached 98.8% on MS basal medium supplemented with 0.25 mg/l thidiazuron+ 0.5 mg/l NAA and 5.0 mg/l $AgNO_3$ in cotyledon explants**.** Chen and Hou [43] reported the regeneration rate of petiole with cotyledon was the highest in the medium containing 0.5mg/l thidiazuron+ 0.5 mg/l NAA+ 7.5 mg/l $AgNO_3$.

Memon et al. [23] reported that low concentration thidiazuron, NAA and $AgNO_3$ is more effective in shoot regeneration per leaf explants than the higher concentration in Chinese cabbage. Whereas, Ravanfar et al. [45] reported very different results that BAP in combination with NAA and 2,4-D are more effective hormones compared with Kn and thidiazuron in broccoli (*Brassica oleracea* L. var. *italica*).

Different auxins i.e. IAA and NAA were used for root regeneration from *in vitro* developed shoots. Hundred per cent root regeneration was achieved in the medium containing 0.10mg/l NAA.High percentage (97.20 %) of root regeneration was also observed on MS medium containing IAA, but MS media containing NAA was most effective. Chen et al. [28] used half strength MS medium containing 0.1mg/l NAA for regeneration of roots. Memon et al. [23] also used different concentration of NAA for rooting and found 88 % root regeneration on medium containing 0.3mg/l NAA. Cheng et al. [41] reported significant increase in the number of roots (about three-fold) formed per explant on the medium containing IAA.The regenerated complete plantlets were transferred to the pots and acclimatized with 80% survival.

A protocol for high frequency plant regeneration via direct and indirect organogenesis has been standardized. This protocol allows the regeneration of cabbage plantlets within 2-3 months and the regenerated plantlets were successfully acclimatized. The present investigation will provide a platform for *in vitro* genetic manipulation.

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