**Momordica dioica** Roxb. (Spine Gourd): Multiple shoot induction from nodal cultures and its antidiabetic activity

Mehul G Patel, Kalpesh B Ishnava

**Abstract**

*Momordica dioica* Roxb. (Spine Gourd) (Cucurbitaceae) is an important vegetable with high food and medicinal value. Maintaining tuber quality in field conditions as well its conservation during storage is difficult. Micropropagation may help to overcome these problems to a great extent. As there is limited information on the above mentioned aspects is not available. Therefore, the present study has been designed to develop an efficient protocol for in vitro shoot multiplication and regenerations of spine gourd as well as to check its antidiabetic activity. The nodal segments were harvested and cut end of the explants then surface sterilized and cultured. Murashige and Skoog’s (MS) agar-gelled medium with optimum concentration of BAP+NAA (1.5+0.1 mg/l) and NAA+NB6 (0.5+0.5 mg/l) had an effect on callus production. Shoot multiplication was found best in NB6 + BAP (0.5+0.5mg/l). After 15 days, shoot length of 5.2 ± 0.37cm and shoot numbers 10±1.4 were observed. In the present investigations, a novel method is developed by which multiple shoot can be induced on MS-medium supplemented with cytokinins (BAP, NAA and NB6). To the best our knowledge this is first report in this *M. dioica* uses the NB6 growth hormones for induction of callus and multiplication of shoot. Antidiabetic activity of *M. dioica* was also evaluated by using α-amylase inhibition assay. Comparative analysis of starch hydrolysis by α-amylase control, fruit and callus extract treated of starch hydrolysis by α-amylase *M. dioica* revealed 3 cm, 2.7 cm and 2.4 cm zone of inhibition respectively. Our result revealed that there is an inhibition α-amylase enzyme responsible for starch hydrolysis. This is in turn helpful to control hyperglycemic condition and type 2 diabetes. However, further study is needed to prove antidiabetic potential of *M. dioica*.

**Keywords:** *Momordica dioica*, Shoot induction, Nodal culture, Antidiabetic activity.

1. **Introduction**

There is a lot of evidence showing that eating plenty of fruits and vegetables is good for our health. It is necessary to investigate those plants which have been used in traditional medicine that have to improve their quality of healthcare. Over the past 30 years, researchers have developed a solid base of science to back up what generations of mothers preached. Vegetables provides a diverse range of tastes, aromas, texture, colours and nutritional attributes, it increase the variety in the food ([Diane et al., 2010](#)) [9]. Vegetables are good source of vitamins, minerals and dietary fiber. Vegetables play a significant role in human nutrition, especially as sources of vitamins C (Ascorbic acid), Thiamine (B1), Niacin (B3), and Pyridoxine (B6), Folacin (also known as folic acid or folate) (B9), minerals and dietary fiber ([Craig and Beck, 1999](#) [7]; [Quebedeaux and Eisa, 1990](#) [23]; [Wargovich, 2000](#) [33]). Vegetables in the daily diet have been strongly associated with reduced risk for some forms of cancer, heart disease, stroke, and other chronic diseases ([Southon, 2000](#) [28]; [Tomas-Barberan and Espin, 2001](#) [30]).

Plant tissue culture technology is being widely used for large scale plant multiplication. Apart from their use as a tool of research, plant tissue culture techniques have in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. Small pieces of tissue can be used to produce hundreds and thousands of plants in a continuous process. Single explants can be multiplied into several thousand plants in relatively short time period and space under controlled conditions, irrespective of the season and weather on a year round basis ([Akin-Idowu et al., 2009](#)) [1]. The micropropagation technology has a vast potential to produce plants of superior quality, isolation of useful variants in well-adapted high yielding genotypes with better disease resistance and stress tolerance capacities ([Brown and Thorpe; 1995](#)) [8].
Momordica dioica belongs to the family Cucurbitaceae. It is a perennial dioecious climber with tuberous root. The genus Momordica is a native of tropical Asia. It is also known as Kakoda (Teasle gourd or Spine gourd). It is used as a vegetable in Odisha, Bengal, Assam and Gujarat in India and some parts in South Asia. It has commercial importance and is exported and used locally. Climbing herbs with tuberous roots; tendrils simple. Leaves 4-8 x 3-6 cm, broadly ovate, deeply 3-5-lobed, base cordate, margin denticulate, apex acute, membranous; petiole to 2.5 cm. Flowers dioecious, solitary, axillary (Figure 1B). Male flowers with long peduncles with memranous; petiole to 2.5 cm. Flowers dioecious, solitary, axillary (Figure 1B). Male flowers with long peduncles with

Mature plant of M. dioica (Kakrol) has a number of problems, including poor natural pollination of female flowers and low yield. Fruits become inedible at maturity owing to the presence of large number of hard seeds. Production of tuberous root pieces per plant is low; 10-20 tuberous pieces are produced per year. Germination of seeds is very difficult or impossible because of hard seed coat. It is impossible to predict sex of seed produced plants before flowering. The plants required artificial transfer of pollen from male to female flower. There is no natural pollinator, hand pollination is necessary. The present study was carried out to develop an efficient protocol for callus induction, proliferation, shoot multiplication using of different growth hormones with nodal explants of M. dioica and also callus extract check their anti diabetic activity.

2. Material and Method

2.1. Plant tissue culture

2.1.1. Explants collection

Mature plant of M. dioica explants (Nodal explants) were collected during June, 2013 from the Napa village and campus nursery of New Vallabh Vidyanagar, District-Anand, Gujarat (Figure 1D). The plants were identified by Dr. Kalpesh Ishnava (Plant taxonomist) at Ashok & Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences (ARIBAS), New Vallabh Vidyanagar, Gujarat, India. The plant materials such as Nodal, leaves and stem collected from one month old plants.

2.1.1.2. Glassware

The glassware used for culture work comprised of Borosil Petridishes, Borosil Sugar Tubes, 100 ml, 250 ml, 500 ml, and 1000 ml corning and Borosil flasks, pipettes, and measuring cylinders (100 ml, 500 ml). Before use, glassware were thoroughly washed with detergent tepool and then washed in running water. These were then treated with Chromic acid (mixture of K2Cr2O7 + H2SO4 + H2O) followed by thorough washing with tap water. The glass wares were then inverted in a clean tray and left to dry in the oven. Plugs for the tubes and flasks were made out of absorbent surgical cotton. 5 - 10 ml water was then poured into every culture vessel which was tightly plugged. The 15 glassware were then steam sterilized in an autoclave at a pressure of 15 psi at 121° C for 15 - 20 minutes.

2.1.1.3. Culture medium

The basal medium used for the culture was Murashige and Skoog medium (Murashige, 1974) with sucrose 3%, 0.8%
agar and Growth hormones (BAP, NB6, KIN, IBA, NAA). The medium was prepared with different combination of growth hormones of different explants of *M. dioica* (Table 1&2) (Murashige, 1974) [20]. The concentrated stock solutions of all the ingredients were prepared and stored under refrigeration. To prepare stock solution of micro salts, all the micro salts in required quantities were dissolved in one liter of distilled water and used as stock solution. Likewise stock solutions of all other ingredients were also prepared and kept under refrigeration. Similarly stock solutions of growth hormones were also prepared. The medium was prepared by adding required quantities of all the ingredients in the conical flask. After adding all the ingredients in required amounts, the final volume is made up with the help of distilled water. pH of the medium is adjusted to 5.8 by using 1N NaOH or 1N HCl. After adjusting the pH, agar is added to the medium at the rate of 0.8% w/v for solidification of the medium. After pouring media (20ml/tube), sugar tubes or bottles are tightly capped and labeled properly. After that media is autoclaved at 121 °C for 20 minutes at 15psi. These were then left to cool and solidify.

### 2.1.1.4. Explants Sterilization

The surface sterilization of different explants (leaves and nodal segments) was carried out in different steps. The nodal explants and leaves were placed in different beaker and covered with net and washed for 30 minutes under running tap water to remove all the adhering dust particles and microbes from the surface, keep all the explants deep into the detergent for 10 minutes then washed with distilled water, then surface sterilize in bavistine (0.1%) for 5 to 7 minutes then washed with distilled water for 2-4 minutes and rinses properly. Then surface sterilized by immersion in streptomycin for 2 minutes, followed by two to four time rinsed for 3 min in sterile distilled water, additionally immersed in 0.1 % mercuric chloride (HgCl) solution for 4 min followed by five time rinses of 5 min each in distilled water. The surface sterilized different explants were blotted dry on sterile filter paper.

### 2.1.1.5. Inoculation of explants

All the experimental manipulations were carried out under strictly aseptic conditions in laminar air flow bench. After sterilization of explants, explants were inoculated in culture bottles aseptically. For inoculation explants were transferred to large sterile glass petriplate or glass plate with the help of sterile forceps under strict aseptic conditions. Here the explants were further trimmed and extra outer leaves were removed to make them in suitable sizes. Trimming and leaves were removed with sterile scalpel blade. After cutting explants into suitable size (for Node), explants are transferred to culture tubes and bottles containing MS medium with NB6, BAP, KIN, IBA of different concentration (Figure 1E). After vertically inoculating the explants in culture tubes the mouth of tube is quick flamed and tubes are tightly capped. After proper labeling clearly mentioning media, date of inoculation etc. the bottles was transferred to growth room.

### 2.1.1.6. Culture condition

The tubes and bottles were shifted to culture room with controlled facility of diffused light (2000 lux) for 10 h daily at 27 ± 2 °C temperatures and 50 to 60% relative humidity.

### 2.2. Multiplications of shoot stage

After 15 days of inoculation culture showing spouting were transferred to full length MS media supplemented with different combination of following hormones concentration for multiplication of shoot regeneration. After cutting explants into suitable size (for Node) of explants are transferred to culture tubes and bottles containing MS medium with NB6, BAP, KIN, IBA of different concentration for shoot multiplication (Table 1). Each sporting nodal segment are individually transfer in the new bottle or sugar tubes for more multiplication shoot regeneration using different concentration of growth regulator. After vertically inoculating the explants in culture tubes the mouth of tube is quick flamed and tubes are tightly capped. After proper labeling clearly mentioning media, date of inoculation etc. the bottles was transferred to growth room.

### Table 1: Effect of MS media and different hormones concentration combination for shoot formation

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Media</th>
<th>Horm Conc. (mg/l)</th>
<th>Average Nodal Length (in cm)</th>
<th>No of Shoots (in cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5th days</td>
<td>10th days</td>
<td>15th days</td>
</tr>
<tr>
<td>1</td>
<td>MS+BAP</td>
<td>0.5</td>
<td>0.4±0.03</td>
<td>1.4±0.23</td>
</tr>
<tr>
<td>2</td>
<td>MS+NB6</td>
<td>0.1</td>
<td>0.5±0.1</td>
<td>1.5±0.5</td>
</tr>
<tr>
<td>3</td>
<td>MS+NB6</td>
<td>0.2</td>
<td>1±0.23</td>
<td>2±0.4</td>
</tr>
<tr>
<td>4</td>
<td>MS+NB6</td>
<td>0.3</td>
<td>0.5±0.2</td>
<td>1.8±0.6</td>
</tr>
<tr>
<td>5</td>
<td>MS+NB6</td>
<td>0.4</td>
<td>0.6±0.3</td>
<td>1.5±0.4</td>
</tr>
<tr>
<td>6</td>
<td>MS+NB6+BAP</td>
<td>0.3±0.3</td>
<td>1.5±0.1</td>
<td>2.8±0.1</td>
</tr>
<tr>
<td>7</td>
<td>MS+NB6+BAP</td>
<td>0.4±0.4</td>
<td>1.2±0.1</td>
<td>2.5±0.1</td>
</tr>
<tr>
<td>8</td>
<td>MS+NB6+BAP</td>
<td>0.5±0.5</td>
<td>1.2±0.1</td>
<td>2.8±0.1</td>
</tr>
<tr>
<td>9</td>
<td>MS+BAP+KI</td>
<td>0.3±0.3</td>
<td>1.4±0.14</td>
<td>3.2±0.14</td>
</tr>
</tbody>
</table>

### 2.3. Anti-Diabetic Activity

#### 2.3.1. Collection of callus

The calluses were collected after 30 to 40 days. Calluses were washed with distilled water to remove all adhering particles of agar. After that calluses were allowed to dry at room temperature.

#### 2.3.2. Extraction of callus

Dried sample of callus from *in vitro* grown plant were grounded to powder with mortar and pestle. Five gram of samples was extracted using 20 ml of methanol in Erlenmeyer flask placed on shaker at 100 rpm for overnight at room temperature. The crude extract then filtered with filter paper (Whatman no. 1). The filtrate was collected and allowed to evaporate. After evaporation the remaining material was collected and different stocks were prepared by dissolving in methanol for amylase screening analysis (starch).

### 2.4. Amylase inhibition screening assay

Requirement of this assay is Alpha amylase, 1% starch, Agar powder, Sodium phosphate buffer (10mM; pH 6.9) and Iodine solution. In this assay, 160 μl of alpha amylase enzyme and 120 μl of plant extract solution were mixed and incubated at 37 °C for 45 min. After incubation, the mixture was poured...
into the well made in the petriplate containing 1% (w/v) agar and 1% (w/v) starch. Plates were allowed to stand for 3 days at 25 °C and then flooded with iodine solution and allowed to stand for 15 min. The diameter of zone of starch hydrolysis was measured. As a control, the enzyme was added into the well of the plate. The % inhibition was calculated by following equation.

\[
\% \text{ Amylase inhibition} = \left( \frac{(\text{diameter of control}) - (\text{diameter of test/diameter of control})}{(\text{diameter of control})} \right) \times 100
\]

3. Result and Discussion

M. dioica belongs to the family Cucurbitaceae. It is annual or perennial climbers. The plant is sometimes found growing wild and is common in hedges. It is often cultivated for its fruits, which are used as vegetable. Teasle gourd is a cucurbitaceous popular summer vegetable. It is available in the forest of dry and moist deciduous in feeding months August to February. Teasle gourd (kankoda) is an important summer vegetable in Gujarat and particularly in north Gujarat part. It is dioecious and propagated vegetatively through tuberous root. In Gujarat, this vegetable plant high economic value with medicinal potential. Improvement of this crop has not been attempted adequately, because of its dioecious nature and its vegetative mode of propagation. Presently its propagation entirely depends on underground tuberous roots, which occupy the valuable cultivable land for a long period i.e. until next planting season. Maintaining tuber quality in field condition as well as to conserve it in storage is difficult. Micropropagation may help overcome these problems to a great extent. So, adequate information on this aspect is not available. Therefore, the present experiment has been designed to develop an efficient protocol for in vitro shoot multiplication and plant regenerations of teasle gourd and to select nodal explants for in vitro shoot multiplication and check the antidiabetic activity.

3.1. Multiplication shoot formation

Effect of BAP on shoot formation

In establishment of the M. dioica shoot formation and elongation observe after 6 days using the BAP (cytokinine) growth hormones. It is best response we get in BAP 0.5 mg/l and after 15 days we get shoot length 2.7±0.30 and shoot no is 2±0.83 observed. In rest of the all combination tubes not observed the response. Nabi et al., (2000) reported the same plant using the nodal explants supplemented with MS medium and growth hormones BAP (1.0 mg/l). In this combination less number of shoots produces. In present study show the more number of shoot produce in the concentration of BAP 0.5 mg/l (Table 1).

Effect of NB6 on shoot formation

In establishment of the M. dioica shoot formation and elongation observe after 5 days as a response to cytokinine. In this study best result observe in NB6 (0.2 mg/l) and after 15 days we get shoot length 4.1±0.66 cm and shoot no observe 3±1.56 (Figure 1F). In other concentrations like 0.1, 0.2 and 0.3 no response observes in KIN. It is first time report in this study best result observed in the 0.3 mg/l BAP + 0.1 mg/1 NAA combination (BAP + KIN) (Table 1). After 15 days shoot length 5.2±0.23 and shoot no observed 3±1.68. In all other tubes contamination occurs.

3.2 Callus induction

Effect of IBA+NAA on callus formation

In establishment of the M. dioica different combination give different result in concentration of IBA+NAA (0.01+ 0.01) observe after 5 day shoot initiation. After 10 days shoot elongation observe and after 15 days increase length of shoot observed. In concentration of IBA+NAA (0.4+0.4), after 5 day shoot initiation observed. After that 10 days callus initiation observe and after 15 days shoot induce from callus (Figure 1G)(Table 2). In other concentration like 0.2, 0.3 and 0.5 no response observed. Ashish (2012) reported the same plant for concentration of IBA+NAA combination observed root initiation with different concentrations of IBA (0.049, 0.148, 0.246, 0.344, 0.443, 0.49μM) and IAA (0.057, 0.171, 0.285, 0.399, 0.514, 0.571 μM). Devendra (2009) reported the excised shoot showed rooting in all treatments, when micro
shoots were inoculated on half strength MS medium supplemented with 1.0mg/l IBA roots emerged within 10-15 days which developed in to good root system after 30 days of culture. A 100 percent of rooting with maximum number of (24.0 ± 0.58) roots per culture with an average (6.5 ± 0.28 cm) root length was observed. The root produced in all the cultures of IBA shows the presence of tuberous roots which is not observed in other cultures containing rooting hormone.

IAA proved as the poor hormone for induction of roots in M. dioica compared to IBA and NAA. The nature of roots formed was thick and long in case of IBA treated cultures. Whereas, thin and stout in both IAA and NAA. The similar results were reported C. sativus (Anup and Bhatnagar, 1995) [3] and M. dioica (Hoque et al., 2000; Nabi et al., 2002)[12, 21]. In our study, observed in this combination is more reposed toward the shoot formation but many reported work it is more reposed toward the root formation. But after long time culture in the growth chamber maintains in this combination IBA + NAA (0.4+0.4) showed the callus, shoot and root formation is observed. After 15 to 20 days callus induction after same callus further subculture for the shoot induction and shoot multiplication is used.

Effect of BAP+NAA on callus formation

In establishment of the M. dioica different combination give different result in concentration of BAP+NAA observed after 5 days callus initiation and after 10 days increase mass of callus and after 15 days white colour callus mass observed (Table 2). Hoque (1995) [13] found that a combination of 1.5 mg/l BAP and 0.1 mg/l NAA was more suitable combination for adventitious multiple shoots formation of M. dioica (Hoque et al., 1995) [13]. Where present investigated BAP+NAA combination is most suitable for callus induction. Devendra (2009) [8] reported that the callus initiation started from nodal base on 10th day 2.0 mg/l NAA, 2.0 mg/l BAP auxin or cytokinins either alone or in combinations were efficient for the induction of callus depending on the varied concentrations of the growth regulators. Callus was observed from leaf explants. Paula et al (1988) [22] reported in Cucumis sativus and in M. dioica reported by Hoque et al., 2000; Nabi et al., 2002[12, 21]. The highest frequencies of callus induction was observed in leaf explants on MS medium supplemented with 1.0 mg/l 2,4-D + BAP 2.0mg/l, followed by node explants in 2.0mg/l NAA + BAP 1.5mg/l with 100 and 80, percent. This study compare to present study more response in the explants is nodal explants.

Effect of NB6+NAA on callus formation

In establishment of the M. dioica in response in combination of NB6+NAA after 5 days callus initiation observe and after 10 days increase mass of callus. After 15 days white colour callus observe (Table 2). NB6 are the more responsible compare to other growth hormones like BAP, IAA and NAA. It play important role in the callus formation. Zhang (2008) [34] reported in Phlox subulata, axillaries as explants, MS media supplemented with 6-benzyoladenine-0.4mg/L; Indole-3-butyric acid - 0.04mg/L and Gibberellins- 1.0mg/L was with highest rate of callus induction of 96%, callus multiplication of 5.6, adventitious buds differentiation of 100%, adventitious buds reproduction of 11.6. In our study observed the long time culture are callus produce in the nodal base of the explants in the combination of NB6+NAA (0.5+0.5mg) (Figure 1H) (Table 2).

### Table 2: Effect of MS media and different hormones concentration combination for callus formation

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Media</th>
<th>Horm Conc. (mg/l)</th>
<th>5th days</th>
<th>10th days</th>
<th>15th days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MS+IBA+NAA</td>
<td>0.01+0.01</td>
<td>Shoot initiation</td>
<td>Shoot initiation</td>
<td>Shoot initiation</td>
</tr>
<tr>
<td>2</td>
<td>MS+IBA+NAA</td>
<td>0.4+0.4</td>
<td>Shoot initiation</td>
<td>Shoot and Callus initiation</td>
<td>Shoot, Callus and Root formation</td>
</tr>
<tr>
<td>3</td>
<td>MS+BAP+NAA</td>
<td>0.5+0.5</td>
<td>Initiation of callus</td>
<td>Increase of callus mass</td>
<td>White colour callus</td>
</tr>
<tr>
<td>4</td>
<td>MS+NB6+ NAA</td>
<td>0.5+0.5</td>
<td>Initiation of callus</td>
<td>Increase of callus mass</td>
<td>White colour callus</td>
</tr>
</tbody>
</table>

### 3.3. Amylase inhibition assay

The inhibition of digestive enzymes, such as α-amylase and α-glucosidase has been considered to be an effective strategy to control blood glucose. Agents based on natural products are particularly attractive as side effects are minimal and the therapies are well-tolerated compared to the other oral hypoglycemic agents currently available. The present study was therefore designed to investigate the bioactive properties of Momordica dioica plants relevant to the management of hyperglycemia and type 2diabetes. These properties included inhibition of α-amylase and α-glucosidase enzymes and antioxidant potential. The present study show the compare to the control (3cm) and fruit (2.7cm) and fresh in vitro callus extracts (2.4cm) less zone of inhibition observed (Table 3 and Figure 1K, 1L and 1M). Inhibition of α-amylase enzyme such that no hydrolysis of starch. In present study less compare to control observed partial. This indicates the present plant alpha amyrase enzyme maximum starch utilized. This indicates in the plate observed the zone of inhibition observed. In our study both fruit and in vitro callus extracts compare to less zone of inhibition. It is revealed the plant extracts amyrase activity is helpful for control the diabetic. Vandana gulati (2012) [31] state that all of the Australian aboriginal plant extracts showed complete inhibition of α-amylase enzyme such that no hydrolysis of starch was evident (Vandana gulati, 2012) [31]. Among the Indian Ayurvedic plant extracts, only Eugenia jambolana and Curculigo orchioides showed complete inhibition at 250 mg/ml. Mucuna pruriens, Boerhaavia diffusa and Pterocarpus marsupium extracts showed partial inhibition. While the majority of the extracts demonstrated potent α-amylase inhibiting activity, some of the Australian plant extracts were particularly active (Acacia ligulata and Acacia tetragonaphylla) and showed activity at concentrations lower than those of the other extracts (Vandana gulati, 2012) [31].

### Table 3: Amylase activity of fruit and fresh callus of Momordica dioica

<table>
<thead>
<tr>
<th>S. No</th>
<th>Extract</th>
<th>Diameter of zone (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>3cm</td>
</tr>
<tr>
<td>2</td>
<td>Fruit</td>
<td>2.7cm</td>
</tr>
<tr>
<td>3</td>
<td>Fresh callus</td>
<td>2.4cm</td>
</tr>
</tbody>
</table>
4. Conclusion
The present study was conducted to establish an effective protocol of plantlet regeneration through shoot multiplication in teak gourd. Shoot multiplication is best response in NB₆ + BAP (0.5 + 0.5mg/l) and (0.3+0.3mg/l) observed. After 15 days shoot length 5.2±0.37 and shoot no observe 10±1.4. In our investigations, a novel method is developed by which multiple shoot can be induced on MS-medium supplemented with cytokinin (BAP, NAA and NB₆). It is first report in this plant used the NB₆ growth hormones for callus induction and multiplication of shoot. The optimum concentration of BAP+NAA (1.5+0.1 mg/l) and NAA+NB₆ (0.5+0.5mg/l) had an effect on callus production. The present investigate the bioactive properties of M. dioica plants relevant to the management of hyperglycemia and type 2diabetes. The present study show the compare to the control (3cm) and fruit (2.7cm) and fresh in vitro callus extracts (2.4cm) less zone of inhibition observed. Inhibition of α-amylase enzyme such that no hydrolysis of starch. In present study less compare to control observed partial. This indicates the present plant α-amylase enzyme starch utilize. It is revealed the plant extracts amylase activity is helpful for control the diabetic.

It is a simple one step protocol for the rapid propagation of underutilize important vegetable crop. Further, this nodal multiplication protocol described may help to develop a useful for the micropropagation for large scale production of plant regeneration from M. dioica. However, further studies are required to provide a detailed phytochemical examination of the active extract of M. dioica to identify the principle(s) responsible for the activity and to elucidate their mechanism of action.

5. Acknowledgements
Authors are thankful to Charutar Vidya Mandal (CVM), Vallabh Vidyanagar and Director of Ashok and Rita Patel Institute of Integrated Studies and Research in Biotechnology and Allied Sciences (ARIBAS), New Vallabh Vidyanagar, Gujarat, India for providing necessary support for research and laboratory facility.

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