Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN 2320-3862
JMPS 2015; 3(6): 82-88

Received: 20-09-2015
Accepted: 21-10-2015

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

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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+NB_6 (0.5+0.5mg/l) had an effect on callus production. Shoot multiplication was found best in NB_6 + 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 NB_6). To the best our knowledge this is first report in this *M. dioica* uses the NB_6 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* reveled 3 cm, 2.7 cm and 2.4 cm zone of inhibition respectively. Our result reveled 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 (B_1), Niacin (B_3), and Pyridoxine (B_6), Folacin (also known as folic acid or folate) (B_9), 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) [6].

It is rapid propagation processes that can lead to the production of plants virus free (Garcia-Gonzales *et al*., 2010) [11]. The past decades of plant cell biotechnology has evolved as a new era in the field of biotechnology, focusing on the production of a large number of secondary plant products. During the second half of the last century the development of genetic engineering and molecular biology techniques allowed the appearance of improved and new agricultural products which have occupied an increasing demand in the productive systems of several countries worldwide (Vasil, 1994) [32]. The commercial application of *in vitro* techniques in cucurbitaceous taxa has been well demonstrated and the regeneration of plants has been reported from excised cotyledons (Gambley and Dodd, 1990 [10]; Singh *et al.,* 1996) [27], leaf explants (Kathal *et al.,* 1988 [14], Mishra and Bhatnagar, 1995 [18]; Stipp *et al*., 2001) [29] and anther culture (Kumar *et al*., 2003) [16].

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 Gujurat 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 foliaceous bracts at apex; peduncle 4-6 cm long. Calyx tube c. 1 cm long, lobes linear, villous. Petals c. 2.5 x 1 cm, oblong, obtuse, yellow. Stamens 3, inserted at the mouth of calyx tube; one 1-celled, two 2-celled; staminodes of 2-3 glands. Ovary oblong with many horizontal ovules. Fruit 2.5 - 6.5 cm long, ellipsoid, shortly beaked, densely echinate with soft spines (Figure 1C). Seeds 6-7 x 5-6 mm, ovoid, emarginate, pale yellow. Flowering and fruiting: July-December.

Fruit of plant are green and generally used as vegetable. It possesses many medicinal properties. Fruit are diuretic, alexiteric stomachic laxative, hepatoprotective, and have antivenom property. It is also used to cure asthma, leprosy, excessive salivation, prevent the inflammation caused by lizard, snake bite, elephantiasis, fever, mental disorders, digestive disorders and troubles of heart and to treat discharge from mucous membrane. Fresh fruit juice is prescribed for hypertension. The fruit is cooked in a small amount of oil and consumed for treating diabetes. Tender fruits are rubbed on skin for pimples and acne. Seeds are roasted and taken for eczema and other skin problems (Sharma, 2004) [26]. The powder or infusion of the dried fruits, when introduced into the nostrils produces a powerful errhine effect and provokes a copious discharge from the schneiderian mucous membrane (Kirtikar and Basu *et al*., 1999) [15].

It is highly nutritionally valuable edible vegetable. Edible portion of the fruit contain moisture – 84.1%, protein – 3.1%, ether extract - 0.97%, carbohydrate – 7.7%, fibre 2.97% and ash 1.1%. It also contains iron 4.6 mg, calcium 33 mg, phosphorus 42 mg, vitamin A 2, thiamine 45.2 mg, riboflavin 176.1 microgram and 0.5 mg/100g. The fruit also contain ascorbic acid. This vegetable and its fruits are used in diabetic treatment prescribe by Ayurvedic practitioners. It is used as an astringent, febrifuge, antiseptic, spermicide and anthelmintic. It possesses antioxidant, antibacterial, anti-inflammatory, antilipid peroxidative, hypoglycemic, and analgesic properties. The fruit plays a role in cures for biliousness, asthma, leprosy, bronchitis, fever, tumors, urinary discharges, excessive salivation and heart disease. Juice of the fruit is a domestic remedy for inflammation. Fruit powder is used to induce sneezing, leading to nasal clearing. Ethanol extracts of fruits has showed nephroprotective activity (Baratakke *et al*. 2013) [5]. Phytoconstituents of *M. dioica* are traces of alkaloids, steroids, triterpenoids, flavonoids, glycosides, saponins (Sadyojatha and Vaidya, 1995) [25]. The alkaloid present in seed called momordicin and present in root called momordicafoetida. Sadyojatha *et al* (1996) [24] examined the chemical constituents of the rhizome of *M. dioica* revealed the presence of β-sitosterol saponin glycosides and alkaloids. Luo *et al* (1997) [17] isolated three triterpenes and two steroidal compounds from dry roots of *M. dioica*. These compounds are alpha-spinasterol octadecanoate (I), alpha-spinasterol-3-O-beta-D-glucopyranoside (II), 3-O-beta-D-glucuronopyranosyl gypsogenin (III), 3-O-beta-D-glucopyranosyl gypsogenin (IV) and 3-O-beta-D-glucopyranosyl hederagenin (V). Ali *et al* (1998) [2] have isolated the two new aliphatic constituents characterized as 6-methyl tritriacont-5-on-28 ol and 8 –methyl hentriacont -3-ene from fruit rind of *M. dioica* along with the known sterol pleuchiol (Ali *et al.,* 1998) [2].

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 antidiabetic 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 brushed with detergent teepol and then washed in running water. These were then treated with Chromic acid (mixture of $K_2Cr_2O_7$ + H_2SO_4+ H_2O) 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) [20] with sucrose 3%, 0.8%

agar and Growth hormones (BAP, NB_6, 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. P^H 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 ($HgCl_2$) 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

Sr. No	Media	Horm Conc. (mg/l)	Average Nodal Length (in cm)			No of Shoots (in cm)		
			5th days	10th days	15th days	5th days	10th days	15th days
1	MS+BAP	0.5	0.4±0.03	1.4±0.23	2.7±0.30	1±1.64	2±0.44	2±0.83
2	MS+NB_6	0.1	0.5±0.1	1.5±0.5	2.5±0.4	1±1.76	2±0.45	2±0.89
3	MS+NB_6	0.2	1±0.23	2.4±0.4	4.1±0.6	1±1.95	2±1.25	3±1.56
4	MS+NB_6	0.3	0.5±0.2	1.8±0.6	3.2±0.6	1±1.89	2±1.54	2±1.67
5	MS+NB_6	0.4	0.6±0.3	1.5±0.4	3.5±0.6	1±1.98	2±1.54	2±1.45
6	MS+NB_6+BAP	0.3+0.3	1.5±0.1	2.8±0.1	4.4±0.2	1±0.7	3±1.5	9±1.3
7	MS+NB_6+BAP	0.4+0.4	1.2±0.1	2.5±0.1	3.1±0.1	1±0.6	2±1.4	3±1.3
8	MS+NB_6+BAP	0.5+0.5	1.2±0.1	2.8±0.1	5.2±0.3	1±0.5	3±1.6	10±1.48
9	MS+BAP+KI	0.3+0.3	1.4±0.14	3.2±0.14	5.2±0.23	1±0.43	2±0.54	3±1.68

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 (10nM; pH 6.9) and Iodine solution. In this assay, 160µl of alpha amylase enzyme and 120 µl of plant extract solution were mixed and incubated at 37 ºC 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 ºC 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.

% Amylase inhibition =
{(diameter of control) - (diameter of test/diameter of control)}

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 vtiro* 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 is 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) [21] 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 NB_6 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 NB_6 (0.2 mg/l). 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, 0.3 and 0.4 good responses but lower than 0.2. NB_6 growth hormone is very closed similar to BAP. Only little bit differences between in their molecular weight. It is very important in the plant tissue culture for shoot initiations. Zhang *et al*., (2008) [34] reported the *Phlox paniculata*, with axillary as explants, basal MS media supplemented with 2.0mg/L 6-benzoyladenine and 0.1mg/L a-Naphthalene acetic acid was the optimum media with 91% budding; MS plus 1.0 mg/L 6-Benzoyladenine and 0.1mg/L a-Naphthalene acetic acid was highest shoot proliferation efficiency of 3.1. It is showed that nodal explants better responded in the NB_6 (0.2 mg/l) compare any other growth hormones (Table 1). It is also observed the after the subculture nodal base produce callus and number of shoot increased early inoculated explants. It is first time report in this plant for NB_6 growth hormones used for shoot formation and multiplication.

Effect of KIN on shoot formation

In establishment of the *M. dioica* no response observes in KIN. In all tubes contaminations occur. Fungal contamination observe due to the not maintaining proper condition or explants sterilization treatment or may be due to endogenous fungus spore present in explants.

Effect of NB_6+BAP on shoot formation

In establishment of the *M. dioica* shoot formation and elongation observe after 3 days as a response to cytokinine (NB_6 + BAP) here in this study best result we get in NB_6 + BAP 0.5 mg/l. After 15 days observe shoot length 5.2±0.37 and shoot no observe 10±1.4 (Table 1) (Figure 1J). In other tube likes 0.3 and 0.4 good response but lower than 0.5 concentrations. In concentration 0.1 and 0.2 we get no response. 0.5 mg/l is the best concentration and higher concentration is good for shoot induction. Only in BAP + NB_6 (0.3+0.3) combination is observe the shoot multiplication. Mohammad and Shorif (2010) [19] reported in the same plant using different concentrations and combinations of BAP and NAA had significant influence on shoot regeneration from shoot tips, internodes, leaf and nodal segments. The results indicated that the nodal explants were more capable of producing multiple shoots compared to other explants. 1.0 mg/1 BAP + 0.1 mg/1 NAA produced shoots in shortest time (15 days) (Figure 1I). Among the various concentrations of NAA at (0.1, 0.2, 0.3) and BAP at (0.5, 1.0, 1.5, 2.0), 1.0 mg1-1 BAP + 0.1 mg1-1 NAA resulted maximum number of shoots from explants and length of longest shoots of explants (0.9 cm) after 30 days from nodal segments (Figure 1I) (Table 1). In present study showed the good result in combination of the growth hormones of BAP + NB_6 (Table 1). It is also shoot number and length increasing. In establishment of the *M. dioica* shoot formation and elongation observe in the combination of BAP + KIN. After 6 days responded in this combination (BAP + KIN) best result observed in the 0.3 mg/l (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) [4] 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) [8] 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 NB_6+NAA on callus formation

In establishment of the *M. dioica* in response in combination of NB_6+NAA after 5 days calls initiation observe and after 10 days increase mass of callus. After 15 days white colour callus observe (Table 2). NB_6 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-benzoyladenine-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 NB_6+NAA **(**0.5+0.5mg) (Figure 1H) (Table 2).

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

Sr. No	Media	Horm Conc. (mg/l)	Observation		
			5th days	10th days	15th days
1	MS+IBA+NAA	0.01+0.01	Shoot initiation	Shoot initiation	Shoot initiation
2	MS+IBA+NAA	0.4+0.4	Shoot initiation	Shoot and Callus initiation	Shoot, Callus and Root formation
3	MS+BAP+NAA	0.5+0.5	Initiation of callus	Increase of callus mass	White colour callus
4	MS+NB_6+ NAA	0.5+0.5	Initiation of callus	Increase of callus mass	White colour callus

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 amylase enzyme maximum starch utilize. 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 amylase 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 tetragonophylla*) 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*

S. No.	Extract	Diameter of zone (cm)
1	Control	3cm
2	Fruit	2.7cm
3	Fresh callus	2.4cm

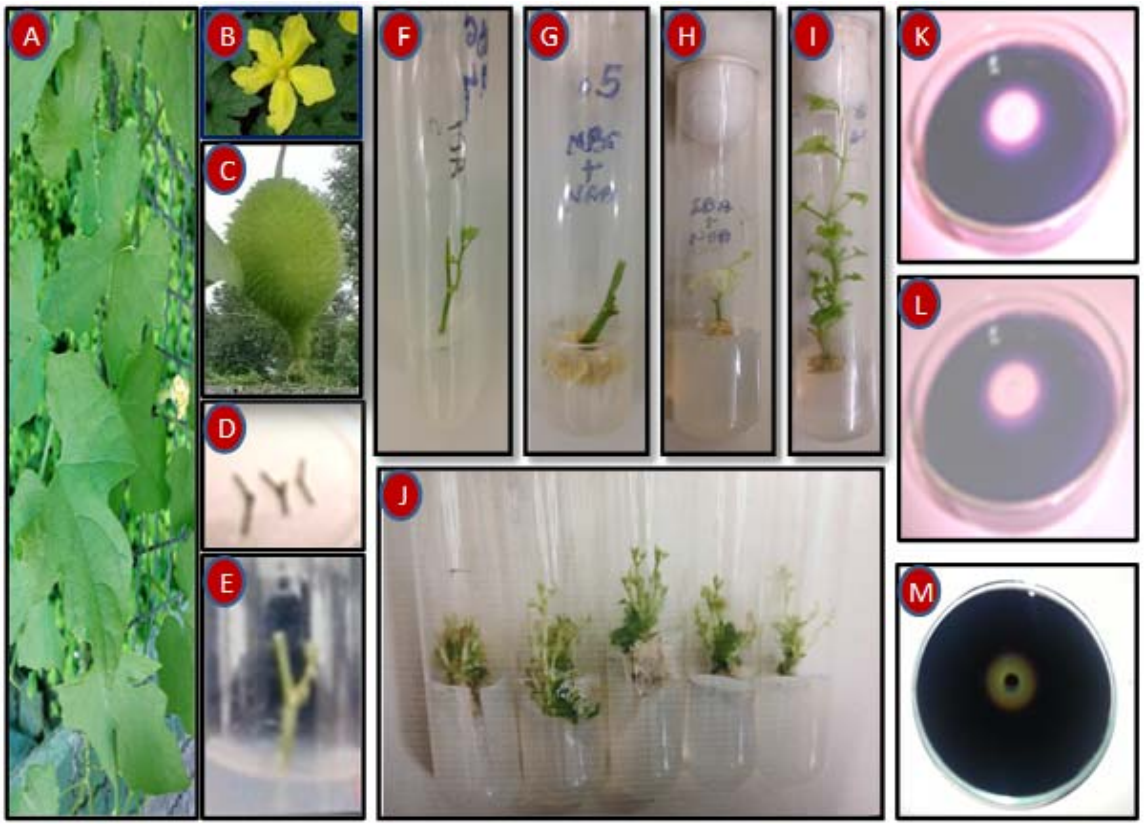

Fig 1: *In vitro* Shoot formation from nodal culture and amylase activity of fruit extract and fresh callus of *Momordica dioica* (A-Whole plant;B-Flower; C-Fruit;D-Nodal Explants; E-Inoculation of nodal explants; F-After 10 days;G-Nodal with callus; H-Shoot and callus; I-Shoot elongation with callus; J- Shoot multiplication; Amylase activity (K- Control; L- Fruit extract; M-Fresh callus)

4. Conclusion

The present study was conducted to establish an effective protocol of plantlet regeneration through shoot multiplication in teasle gourd. Shoot multiplication is best response in NB_6 + 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 NB_6). It is first report in this plant used the NB_6 growth hormones for callus induction and multiplication of shoot. The optimum concentration of BAP+NAA (1.5+0.1 mg/l) and NAA+NB_6 (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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