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Research Article

Development of *In Vitro* Regeneration Protocol for Sweet Pepper (*Capsicum annuum* L.) using Cotyledon as Explant

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ABSTRACT

Plant tissue culture techniques offer a promising avenue for the efficient propagation of sweet pepper (*Capsicum annuum* L.), a valuable crop with significant agricultural importance. An effective and repeatable *in vitro* plant regeneration protocol was developed for the CKN-1 (Lemon yellow) and CKN-8 (Red) genotypes of sweet pepper. Utilizing cotyledonary leaf and cotyledonary node explants, various concentrations and combinations of growth regulators were tested for shoot rejuvenation. For both explants, MS medium supplemented with 8 mg/L BAP, 0.02 mg/L NAA, and 0.5 mg/L IAA was found to be most effective for achieving high rates of response and shoot induction. Notably, cotyledonary node explants outperformed cotyledonary leaf explants, displaying a remarkable 80% response within 11-12 days. Additionally, the supplementation of 1 mg/L IBA with MS medium significantly improved root initiation, with cotyledonary node explants exhibiting the highest responsiveness at 83.77%. Throughout all stages, the genotype CKN-8 (Red) consistently outperformed CKN-1 (Lemon yellow), including in the percentage of regenerated plant establishment.

Introduction

Sweet peppers are the edible fruits of plants from the *Capsicum* genus of the Solanaceae family. The genus *Capsicum* has around 25 wild and 5 adapted species. *Capsicum annuum* L., *Capsicum chinense* Jacq., *Capsicum frutescens* L., *Capsicum baccatum* L., and *Capsicum pubescens* R and P, are the five adapted species (IBPGR, 1983). *Capsicum annuum* holds the utmost economic importance among all species, and it produces both mild and spicy fruits. *Capsicum annuum*, sometimes known as sweet pepper, pepper, or bell pepper is a widely grown vegetable crop worldwide. Many chemicals, such as steam-volatile oil, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fiber, and mineral elements, are found in sweet peppers (Bosland and Votava, 2000). It is high in vitamins A, B-complex, C, and E, as well as minerals such as molybdenum, manganese, phosphorus, and potassium. Because of their nutritional value, flavor, aroma, texture, pungency, and color, sweet peppers are used in a wide variety of foods, medications, and cosmetics (Shu *et al*., 2022; Saleh *et al*., 2018). They are also grown for their beautiful glossy fruits, which come in a variety of sizes, shapes, and colors including red, green, yellow, brown, and purple. They can be used as a vegetable, condiment, or spice to add color, taste, zest, and piquancy to many cuisines. Capsicum typically a summer crop in temperate countries, although sweet pepper is currently being widely grown in Bangladesh (Mahmud *et al.,* 2017). Although some advanced farmers sporadically cultivate capsicum, their production falls short of meeting the increasing demand from those residing in rural areas. (Saha and Salam, 2004).

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Pepper production is limited due to its susceptibility to infections such as viruses, fungus, bacteria, and nematodes, as well as harsh temperatures (Pochard *et al.*, 1992). Moreover, pepper seeds often have a limited viability period and quickly lose their ability to germinate (Mezghani *et al.*, 2007). Tissue culture for plant propagation offers numerous advantages compared to conventional methods. These include accelerated multiplication of valuable genotypes, quicker introduction of improved cultivars, production of disease-free plants, year-round cultivation, preservation of germplasm, and simplified exchange of these genetic materials (Sanatombi and Sharma, 2008). However, because of the genus Capsicum's recalcitrance to *in vitro* regeneration, genetic transformation using recombinant DNA technology is becoming challenging or ineffective (Ashrafuzzaman *et al.*, 2009). Several investigations for *in vitro* regeneration of sweet peppers have been conducted both internationally and in Bangladesh. Numerous experiments were carried out to demonstrate that the success of plant micro-propagation in different pepper species (*Capsicum* spp.) is influenced by factors such as plant genotype, type of initial explants, and the parameters and conditions of *in vitro* cultures (Dabauza and Pena, 2001).

The organogenic response of *in vitro* cultures in various plant species is significantly influenced by genotype, making it a crucial determinant. Pepper genotypes exhibit notable variations in their ability for organogenesis (Christopher and Rajam, 1994), a trait also observed across different cultivars and species (Ezura *et al*., 1993; Szasz *et al*., 1995; Ramirez and Ochoa, 1996). In tissue culture, plant regeneration heavily relies on the interaction between auxin and cytokinin hormones. Cytokinin, particularly 6-Benzylaminopurine (BAP), plays a pivotal role in promoting shoot organogenesis by stimulating cell division and shoot proliferation. Meanwhile, synthetic auxin hormones like Naphthalene Acetic Acid (NAA) and indole-3-acetic acid (IAA) facilitate root formation and regulate various growth processes respectively (Hill and Schaller, 2013). Successful techniques for pepper regeneration often involve inducing shoot-bud organogenesis from cotyledon and hypocotyl tissues, a method widely documented across multiple cultivars (Fari and Czako, 1981; Sripichitt *et al*., 1987; Subhash and Prolaram, 1987; Agrawal *et al*., 1988; Subhash and Christopher, 1988; Agrawal *et al*., 1989; Jacobs and Stephens, 1990; Arroyo and Revilla, 1991; Wang *et al*., 1991). These findings underscore the genotype-dependent nature of organogenic responses and the pivotal roles of auxin and cytokinin in plant tissue culture.

This research aimed to establish an efficient and replicable *in vitro* regeneration protocol of two sweet pepper (*C. annuum*) genotypes, CKN-1 (lemon yellow) and CKN-8 (red), for the development of transgenic capsicum lines.

Materials and Methods

The study was carried out at the Genomics Laboratory within the Biotechnology Division of the Bangladesh Institute of Nuclear Agriculture (BINA), situated on the BAU campus in Mymensingh, Bangladesh, during July to December 2023. The research focused on two genotypes, CKN-1 (lemon yellow,) and CKN-8 (red), colleced from the Asian Vegetable Research and Development Center (AVRDC), Taiwan.

Seed Treatment and Germination

Seeds of CKN-1 (lemon yellow) and CKN-8 (red) underwent a series of treatments to enhance germination. These treatments included washing with 2% (v/v) Tween-20 for 5 minutes, followed by soaking in distilled water at room temperature and 4°C for two days. Additionally, seeds were submerged in distilled water containing 2 mg/L gibberellic acid (GA_3) under similar conditions. The process involved in preparing fresh seeds included sterilization through immersion in 70% ethanol for 1 minute, followed by exposure to 0.1% mercuric chloride ($HgCl_2$) for 2 minutes. Afterward, they were rinsed five times with sterile double distilled water and then placed onto half-strength solid MS basal medium under aseptic conditions. Incubation took place at a temperature of 25±2°C, with a photoperiod of 16 hours of light and 8 hours of darkness, to facilitate seedling growth.

Explant Harvesting and Shoot Initiation

Five to seven-day-old seedlings were utilized to harvest cotyledonary explants (cotyledonary leaves and nodes), measuring from 0.4 to 0.6 cm. These tissue samples were horizontally placed onto MS basal medium enriched with 3% sucrose, 0.8% agar, and varying concentrations of growth regulators including BAP (4, 6, 8 mg/L), NAA (0.02 mg/L), and IAA (0.5, 1.0 mg/L) to obtain the best yielding combination for subsequent plant regeneration . Gelrite was used as the solidifying agent for these rooting media and the pH was adjusted to 5.8. The Petri dishes holding the tissue samples were sealed using parafilm strips to safeguard against contamination. Subsequently, the cultures were placed in an incubator set at 25±2°C, with a light cycle of 16 hours of illumination followed by 8 hours of darkness.

Root Induction and Acclimatization

The elongated individual shoots obtained from the shooting medium were separated and relocated to test

tubes containing MS medium enriched with varying concentrations of indole-3-butyric acid (IBA) - 0.25 mg/L, 0.5 mg/L, and 1.0 mg/L - to stimulate root formation (Akther *et al.*, 2020). Following root induction, the plantlets were delicately extracted from the test tubes, cleansed to eliminate any remaining medium, and transplanted into small plastic pots filled with sterilized soil for establishment and adaptation under controlled conditions. Each pot was shielded with a polythene bag to sustain humidity and positioned in a culture room set at 25±2°C, with a light schedule of 16 hours of illumination and 8 hours of darkness. After 6-7 days, the polythene bags were removed, and after 14 days, the plantlet-containing pots were transferred to larger pots containing a mixture of soil, sand, and farmyard manure (in a ratio of 1:1:1). Subsequently, the pots with the plantlets were relocated to outdoor natural conditions to facilitate further growth.

Data Collection and Analysis

Various parameters including responsive explants percentage, days to shoot initiation, shoot regeneration percentage, root induction percentage, and survival rate of regenerated plants were assessed. Observations were recorded at different stages of the regeneration process. Data were analyzed statistically using R software and MS Excel following a Completely Randomized Design. Triplicate data from various treatments were utilized to calculate mean and standard errors. LSD values were calculated at 5% level of significance. Statistical analysis was conducted to assess the effectiveness of different hormone combinations and concentrations in promoting in vitro regeneration of sweet pepper plants.

Results and Discussion

In vitro-based breeding strategies encounter challenges due to the inherent difficulty in regenerating peppers (*Capsicum* spp.) through tissue culture techniques. Furthermore, there is variation in the regeneration response among species and types of explants (Martínez-Lopez *et al.*, 2021). The plant hormones auxin and cytokinin play essential roles in plant regeneration through tissue culture, with cytokinin being particularly pivotal in enhancing shoot initiation (Hill and Schaller, 2013). Different levels of BAP (6-benzylaminopurine), IAA, and NAA (α-naphthaleneacetic acid) were utilized in MS medium to determine the most optimal combination for initiating and fostering shoot growth from both types of explants, namely cotyledonary leaf and cotyledonary node, across different genotypes. The outcomes for both genotypes, CKN-1 and CKN-8, are detailed in Table 1 and Table 2. The percentage of responsive explants and the days taken for shoot initiation were recorded for each combination.

Table 1. The responses of explants to shoot regeneration on MS medium with diverse combinations of BAP and IAA for the CKN-1 genotype

Explant	PGR (mg/L)		Number of explants inoculated	Responsive explants (%)	Time Taken for Shoot Initiation (Days)	Developed shoots (%)
	BAP	IAA				
CL	4	0.5	40	30.83 f	27-28	20.29 i
	4	1.0	40	38.33 ef	27-28	23.62 hi
	6	0.5	40	34.16 f	25-27	30.17 g-i
	6	1.0	40	41.67 ef	25-26	37.69 d-f
	8	0.5	40	51.67 cd	22-23	46.48 b-e
	8	1.0	40	44.17 c-e	22-23	39.27 e-g
CN	4	0.5	40	41.67 de	15-17	45.32 f-h
	4	1.0	40	50.83 bc	15-16	50.54 c-f
	6	0.5	40	46.67 cd	14-16	60.93 ab
	6	1.0	40	58.33 a	13-15	69.73 a-d
	8	0.5	40	65.83 a	11-12	73.49 a
	8	1.0	40	51.67 ab	11-12	69.34 a-c
LSD (0.05)				10.46		5.83

CL= Cotyledonary leaf, and CN= Cotyledonary node

Table 2. The responses of explants to shoot regeneration on MS medium with diverse combinations of BAP and IAA for the CKN-8 genotype

Explant	PGR (mg/L)		Number of explants inoculated	Responsive explants (%)	Time Taken for Shoot Initiation (Days)	Developed shoots (%)
	BAP	IAA				
CL	4	0.5	40	35.83 g	27-28	14.69 g
	4	1.0	40	43.33 e-g	27-28	16.67 g
	6	0.5	40	36.67 fg	25-27	17.24 fg
	6	1.0	40	42.50 d-f	26-27	23.93 ef
	8	0.5	40	58.33 bc	21-23	26.85 de
	8	1.0	40	50.83 c-e	21-22	22.78 d-f
CN	4	0.5	40	48.33 d-f	15-17	20.59 de
	4	1.0	40	60.83 b-d	15-16	25.39 cd
	6	0.5	40	57.50 c-e	13-14	31.87 bc
	6	1.0	40	73.33 ab	13-14	29.34 ab
	8	0.5	40	80.00 a	11-12	33.28 a
	8	1.0	40	70.83 bc	11-12	31.05 ab
LSD (0.05)				9.26		11.52

CL= Cotyledonary leaf, and CN= Cotyledonary node

In both Table 1 and 2, increasing concentrations of both BAP and IAA were found to generally correlate with higher percentages of responsive explants and enhanced shoot regeneration for both types of explants. However, specific details differed between the two tables. In the case of cotyledonary leaf explants, Table 1, representing the CKN-1 genotype, demonstrated the highest response rates, reaching 51.67%, with a shoot regeneration rate of 46.48% when exposed to 8 mg/L BAP and 0.5 mg/L IAA. Conversely, Table 2, representing the CKN-8 genotype, exhibited slightly elevated response rates of 58.33%, accompanied by a lower shoot regeneration rate of 26.85% under identical conditions. In contrast, cotyledonary node explants consistently outperformed cotyledonary leaf explants in both tables. In Table 1, the highest response rates were observed at 65.83%, accompanied by shoot regeneration reaching 73.49% when exposed to 8 mg/L BAP and 0.5 mg/L IAA. Similarly, Table 2 displayed even higher response rates, reaching 80.00%, with shoot regeneration recorded at 33.28% using the same concentrations of BAP and IAA. Moreover, both tables noted a decrease in the time needed for shoot initiation as the concentrations of BAP and IAA increased, suggesting a likely dose-dependent impact on shoot initiation. Overall, the concentrations of both BAP and IAA significantly influenced shoot regeneration from cotyledonary leaf and cotyledonary node explants of both genotypes, with higher concentrations generally resulting in increased percentages of responsive explants and faster shoot initiation. Interestingly, the highest response and shoot initiation were observed at 0.5 mg/L IAA rather than 1.00 mg/L IAA, potentially due to the combined effect of BAP, NAA, and IAA. However, Ebida and Hu (1993) observed similar outcomes, highlighting the most effective BAP to NAA combinations at 5.0 mg/L : 0.1 mg/L for shoot regeneration from cotyledon explants of *Capsicum annuum*. Robinson and Maheswari (2013) also documented improved shoot production from nodal explants of the kandhari variety of *Capsicum annuum* using MS medium supplemented with 5 mg/L BAP.

The root induction percentages were documented for each combination of media and IBA concentration (Figure 1). It is apparent for both CKN-1 and CKN-8 genotypes that the inclusion of IBA significantly boosts root induction compared to the control (MS+ No Hormone). Across all IBA concentrations (0.25 mg/L, 0.50 mg/L, and 1.0 mg/L), there is a consistent uptick in the root induction percentage for both cotyledonary leaf and cotyledonary node explants. Cotyledonary node explants consistently exhibited higher root induction percentages compared to cotyledonary leaf explants across all IBA concentrations. In the case of CKN-1, cotyledonary node explants demonstrated the highest root initiation at 77.51%, while for cotyledonary leaf, it was 61.67%, with MS supplemented with 1.0 mg/L IBA. Similarly, concerning CKN-8, the combination of MS with 1.0 mg/L IBA yielded the highest root induction percentages, 83.77% and 66.37% for cotyledonary node and cotyledonary leaf, respectively. This implies that cotyledonary node explants exhibit more responsiveness to IBA-induced root induction compared to cotyledonary leaf explants in both CKN-1 and CKN-8 genotypes. Furthermore, CKN-8 displayed higher root induction percentages compared to CKN-1 across all IBA concentrations and explant types. Ahmad *et al*. (2006) successfully regenerated shoots from cotyledon explants of *Capsicum annuum*, achieving a 90% root formation frequency in a medium containing 1.0 mg/L. Hegde *et al*. (2017) regenerated shoots from cotyledon explants cultured on MS media supplemented with 0.5 mg/L IBA and observed 100% rooting in two hybrids.

Whiskers of the bolxplot exhibits the highest and lowest percentages of established plants from cotyledonary leaf and cotyledonary node explants of CKN-1 and CKN-8 genotypes of *Capsicum annuum* across multiple replications (Figure 4). The error bars represents variability or standard deviation around the mean and median. Among 72 samples, CKN-8 showed higher percentages of established plants compared to CKN-1 for both cotyledonary leaf (56.0%) and cotyledonary node (74.2%) explants. Within each genotype, cotyledonary node explants tend to yield higher percentages of established plants compared to cotyledonary leaf explants. Previous studies have similarly reported that, *in vitro* regeneration of sweet pepper plants highly depends on the genotype and explant type (Hegde *et al.*, 2017; Dabauza and Pena, 2001).

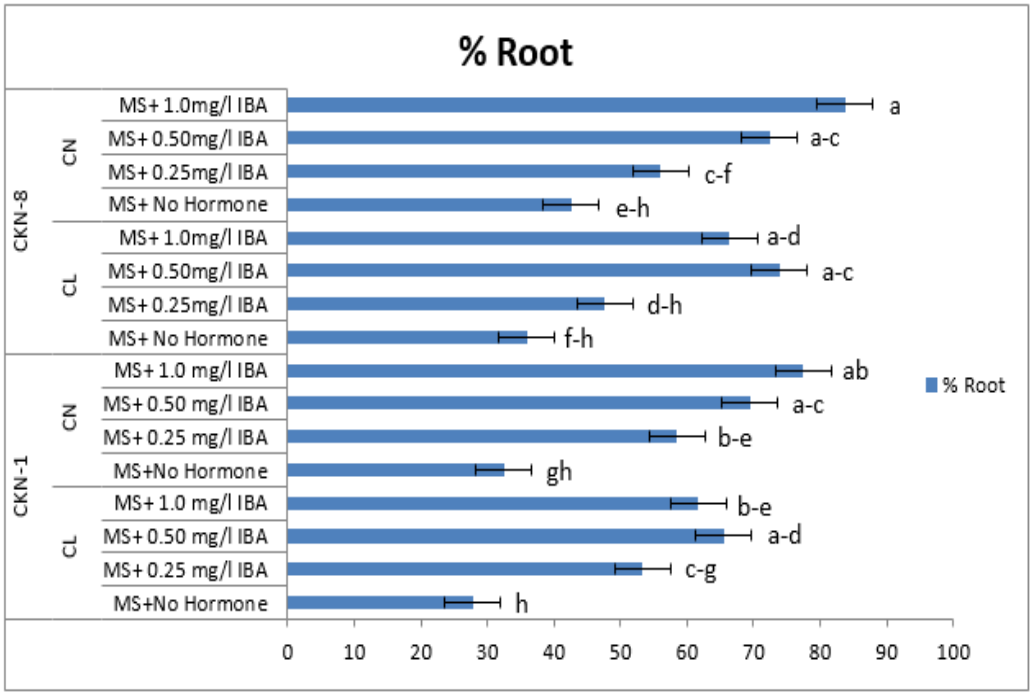

Figure 1. Influence of various growth media and plant growth regulator (IBA) conditions on root induction from *in vitro* regenerated shoots of CKN-1 and CKN-8 genotypes of *Capsicum annuum* (CL= Cotyledonary leaf, CN= Cotyledonary node)

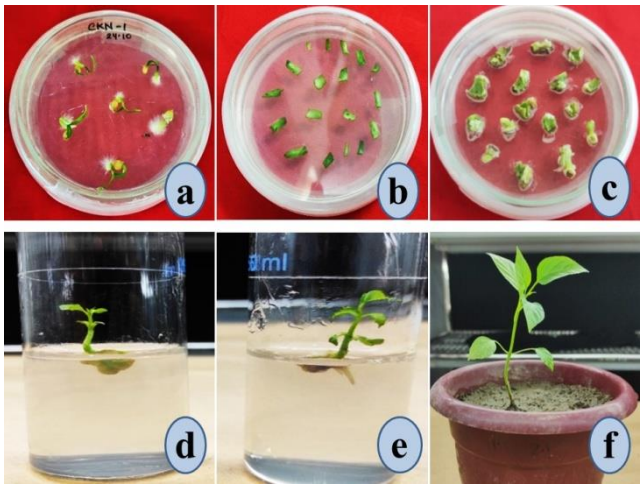

Figure 2. Various phases of regeneration from cotyledonary leaf explants of both CKN-1 and CKN-8 genotypes of *Capsicum annuum*. (a) Germination and plantlet development from seeds, (b) Cotyledonary leaf explants cultured on MS media with different combinations of BAP, NAA, and IAA, (c) Initiation of direct shoot from cotyledonary leaf explants on the same media combination, (d) Elongated shoot development from cotyledonary leaf explants, (e) Root induction from the elongated shoot, (f) Established plant in a pot.

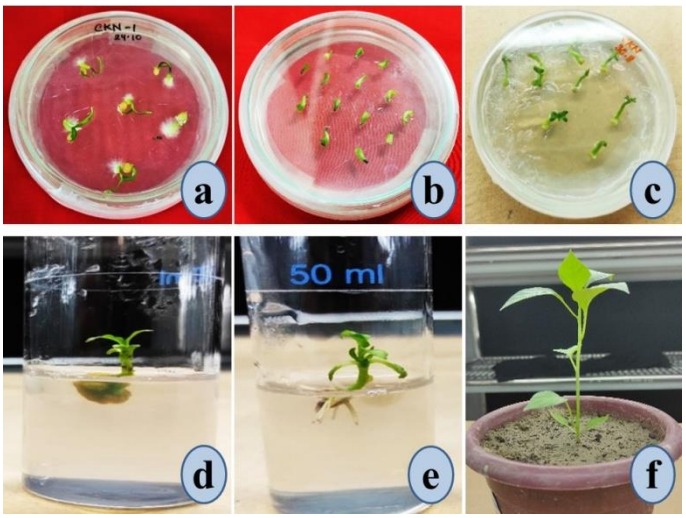

Figure 3. Various phases of regeneration from cotyledonary node explants of both CKN-1 and CKN-8 genotypes of *Capsicum annuum*. (a) Germination and plantlet development from seeds, (b) Cotyledonary node explants cultured on MS media with different combinations of BAP, NAA, and IAA, (c) Initiation of direct shoot from cotyledonary node explants on the same media combination, (d) Elongated shoot development from cotyledonary node explants, (e) Root induction from the elongated shoot, (f) Established plant in a pot.

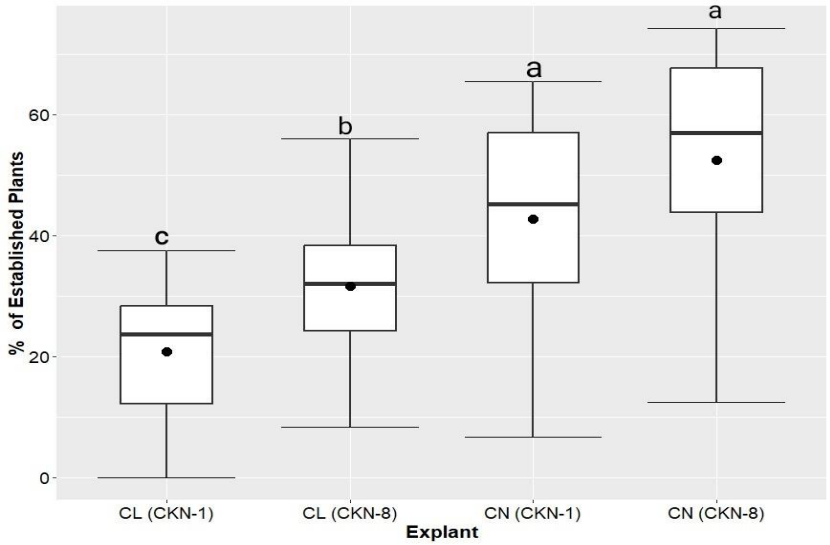

Figure 4. Influence of the type of explant and genotype on the percentage of successfully established plants of *Capsicum annuum* (CL= Cotyledonary leaf, CN= Cotyledonary node).

Conclusion

A successful optimization of an effective and reproducible direct *in vitro* regeneration protocol was achieved for two sweet pepper (*Capsicum annuum*) genotypes. This was achieved by employing cotyledonary node and cotyledonary leaf explants, with the cotyledonary node proving to be more effective in inducing both shoot and root formation. This protocol will contribute to the development of efficient breeding strategies and transgenic lines in sweet pepper.

References

Ahmad, N., Siddique, I., Anis, M. (2006). Improved plant regeneration in *Capsicum annuum* L. from nodal segments. Biologia Plantarum, 50: 701-704.

Agrawal, S., Chandra, N., Kothari, S. L. (1989). Plant regeneration in tissue cultures of pepper (*Capsicum annuum* L. cv. Mathania). Plant cell, tissue and organ culture, 16: 47-55.

Agrawal, S., Chandra, N., Kothari, S. L. (1988). Shoot tip culture of pepper for micropropagation. Current Science, 57(24): 1347-1349.

Akther, S., Banu, T. A., Khan, S., Akter, S., Habib, A., Islam, M., Goswami, B., Sarkar, B. K. (2020). Micropropagation of two varieties of bell pepper (*Capsicum annuum* L.). Plant Tissue Culture and Biotechnology, 30(2): 267–275.

Arroyo, R., Revilla, M. A. (1991). *In vitro* plant regeneration from cotyledon and hypocotyl segments in two bell pepper cultivars. Plant Cell Reports, 10: 414-416.

Ashrafuzzaman, M., Hossain, M., Razi, M., Haque, M.S. (2009). Regeneration potential of seedling explants of chilli (*Capsicum annuum*). *African Journal of Biotechnology*, 8(4): 591-596.

Bosland, P. W., Votava, E.J. (2000). Peppers: Vegetables and Spice Capsicums. Crop Production Science in Horticulture 12.CAB International Publishing, Wallingford, England, UK.p: 204 .

Christopher, T., Rajam, M.V. 1994. Invitro clonal propagation of *Capsicum spp*. Plant Cell, Tissue and Organ Culture, 38: 25-29.

Dabauza, M., Pena, L. (2001) High efficiency organogenesis in sweet pepper (*Capsicum annuum* L.) tissue from different seedling explants. Plant Growth Regulation, 33: 221-229. https://doi.org/10.1023/A:1017585407870

Ebida, A. I., Hu, C. Y. (1993). In vitro morphogenetic responses and plant regeneration from pepper (*Capsicum annuum* L. cv. Early California Wonder) seedling explants. Plant cell reports, 13(2): 107–110.

Ezura, H., Nishimiya, S., Kasumi, M. (1993). Efficient regeneration of plants independent of exogeneous growth regulators in bell pepper (*Capsicum annuum* L.). Plant Cell Reports, 12(12): 676-680.

Fari, M., Czako, M. (1981). Relationship between position and morphogenetic response of pepper hypocotyl explants cultured *in vitro*. Scientia Horticulturae, 15(3): 207-213.

Hegde, Vivek, Partap, P.S., Yadav, R.C. (2017). *In vitro* regeneration of capsicum (*Capsicum annuum* L.) from cotyledon explants. *International Journal of Current Microbiology and Applied Sciences*, 6: 225-237.

Hill, K., Schaller, G.E. (2013). Enhancing plant regeneration in tissue culture: a molecular approach through manipulation of cytokinin sensitivity. Plant signaling and behavior, 8(10): 10.4161/psb.25709.

IBPGR (1983). Genetic resources of Capsicum. International Board for Plant Genetic Resources, Rome.

Jacobs, J. L., Stephens, C. T. (1990). Factors affecting the regeneration of pepper (*Capsicum annuum* L.). HortScience, 25(9): 1120G-1120.

Mahmud, N., Chakma, R., Ahmed, N., Zaman, M., Hossain, A. (2017). Effect of sowing date on quality seed production of sweet pepper in Bangladesh. Progress. Agriculture, 28(3): 216-221.

Martinez-Lopez, M., Garcia-Perez, A., Gimeno-Paez, E., Prohens, J., Vilanova, S., Garcia-Fortea, E (2021). Screening of Suitable Plant Regeneration Protocols for Several *Capsicum spp*. through Direct Organogenesis. Horticulturae, 7: 261.

Mezghani, N., Gammali, A., Elloumi, N., Gargouri-Bouzid, R., Kintzios, S. (2007). Morpho-histological study on shoot bud regeneration in cotyledon cultures of pepper (*Capsicum annuum* L.). Biologia Bratislava, 62(6): 704-710.

Pochard, E., Palloix, A., Daubeze, M. (1992). In: Amelioration des Especes Vegetales Cultivees, Objectifset Criteres de Selection, INRA, Paris.Le piment, pp. 420– 434.

Robinson, J. P., Maheswari, M. (2013). *In vitro* multiple shoot induction from nodal explants of *Capsicum annuum* L. of kandhari variety. *International Journal of Current Microbiology and Applied Sciences*, 2(6): 57-63.

Saleh, B. K., Omer, A., Teweldemedhin, B. J. M. F. P. T. (2018). Medicinal uses and health benefits of chili pepper (*Capsicum spp*.): a review. MOJ Food Processing and Technology, 6(4): 325-328.

Sanatombi, K., Sharma,G,S. (2008). *In vitro* plant regeneration in six cultivars of *Capsicum spp*. using different explants. Biologia plantarum, 52(1):141-145.

Shu, H., Zhang, Y., He, C., Altaf, M. A., Hao, Y., Liao, D., Li, L., Li, C., Fu, H., Cheng, S., Zhu, G., Wang, Z. (2022). Establishment of *in vitro* regeneration system and molecular analysis of early development of somatic callus in *Capsicum chinense* and *Capsicum baccatum*. Frontiers in plant science, 13: 1025497.

Sripichitt, P., Nawata, E., Shigenaga, S. (1987). *In vitro* shoot-forming capacity of cotyledon explants in red pepper (*Capsicum annuum* L. cv. Yatsufusa). *Japanese Journal of Breeding*, 37(2): 133-142.

Subhash, K., Christopher, T. (1988). Direct plantlet formation in cotyledon cultures of *Capsicum frutescens*. Current Science, 57: 99–100.

Subhash, K., Prolaram, B. (1987). Induction of multiple shoots by gamma rays from cotyledon cultures of Capsicum. Current Science, 56(24): 1289-1291.

Szasz, A., Nervo, G., Fari, M. (1995). Screening for in vitro shoot-forming capacity of seedling explants in bell pepper (*Capsicum annuum* L.) genotypes and efficient plant regeneration using thidiazuron. Plant cell reports, 14: 666-669.

Wang, Y. W., Yang, M. Z., Pan, N. S., Chen, Z. L. (1991). Gus expression in hypocotyls and cotyledons of transformed sweet paper. Acta Botanica Sinica, 33: 780-786.