Cross Current International Journal of Agriculture and Veterinary Sciences
Abbreviated Key Title: Cross Current Int J Agri Vet Sci
ISSN: 2663-2454 (Print) & Open Access
DOI: 10.36344/ccijavs.2021.v03i05.001

Volume-3 | Issue-5 | Aug, 2021 |

Original Research Article

Anther Culture Response of Selected Genotypes of Capsicum (*Capsicum annuum* L)

D.M.H. Ranasinghe[1], H.M.P.S. Kumari[2] and P.A. Weerasinghe[1*]

[1]Department of Plant Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka, Anuradhapura, Sri Lanka
[2]Horticultural Crops Research and Development Institute, Department of Agriculture, Gannoruwa, Peradeniya, Sri Lanka

***Corresponding author:** P.A. Weerasinghe

| **Received:** 11.07.2021 | **Accepted:** 14.08.2021 | **Published:** 20.08.2021 |

Abstract: The experiment was conducted to study anther culture response in selected genotypes of Capsicum (*Capsicum annuum* L.) with the objectives of finding suitable callus induction and regeneration media to speed up the plant breeding programme. The research was designed in Completely Randomized Design. Two different callus induction media were used with different concentrations of NAA 5 mgL^{-1} (CI-1) and 2.5 mgL^{-1} (CI-2) on MS medium to find out the best medium for callus induction using Capsicum anthers. The highest percentage of callus induction (52.17%) was obtained in 2.5 mgL^{-1} NAA (CI-2). To compare the best capsicum variety for callus induction, anthers of one inbred line (1782) and six F1 hybrid varieties (HORDI CAH-43, HORDI CAH-44, HORDI CAH-45, HORDI CAH-46, HORDI CAH-47, HORDI CAH-48) were cultured on CI-2 medium which presented the best callus induction. There was a significant effect in variety-wise comparison ($p<0.05$) on anther callus induction. The HORDI CAH-45 was given the highest percentage of callus induction (59.32%). Selected calli from the above experiment were transferred into six different callus regeneration media of 1.5, 2.0 and 2.5 mgL^{-1} Kinetin, and 1.5, 2.0 and 2.5 mgL^{-1} Glycine to find out the suitable callus regeneration medium. Callus enlargement and greening had significant ($p<0.05$) effects on different concentrations of Kinetin and Glycine media. The highest percentage of callus enlargement (99.25%) was obtained in 2.0 mgL^{-1} Glycine from HORDI CAH-43 and the highest percentage of greening (88.89%) was obtained in 2.0 mgL^{-1} Kinetin from HORDI CAH-45. It can be concluded that HORDI CAH-45 F1 hybrid variety was the best for callus induction in the MS medium supplemented 2.5 mgL^{-1} NAA and for the greening of callus in the MS medium combined with 2.0 mgL^{-1} Kinetin. The best callus enlargement was obtained in MS medium supplemented with 2.0 mgL^{-1} Glycine from HORDI CAH-43.

Keywords: Anther culture, Capsicum, Callus induction, Regeneration.

INTRODUCTION

Capsicum annuum L. is in family Solanaceae and genus Capsicum, native to southern North America and northern South America. The genus Capsicum includes twenty-six wild species and five domesticated varieties [1].

Only a few high-yielding local varieties are available in Capsicum. The genetic variability of this crop is broad and has the potential to generate outstanding varieties. Breeding programs can increase the production of newly improved varieties with desirable characteristics. With conventional breeding, the new variety development procedure takes more than 6 years. There is a big growing demand to improve this species due to their profitability and export demand [2].

Haploid plants which emerge from pollen is a new aspect to plant breeding. Haploid cultures are also important for experimental material as genetic, cytological, and biochemical studies. Haploid individuals are useful for genetic engineering purposes and can easily be visible in induced mutations [3]. Anther culture was the first discovered haploid inducing and its efficiency was sufficient for plant breeding purposes [4]. The first report for induction of callus in anther culture of *Capsicum annuum* L. was published in 1973 by [3].

The best method of obtaining haploid plants in Capsicum is androgenesis [5]. The number of factors influence the effectiveness of haploid induction. They are the genotype of the donor plant, developmental

Quick Response Code

Journal homepage:
http://crosscurrentpublisher.com

Citation: D.M.H. Ranasinghe *et al* (2021). Anther Culture Response of Selected Genotypes of Capsicum *(Capsicum annuum* L*). Cross Current Int J Agri Vet Sci, 3*(5), 47-53.

stage of microspores, and culture maintenance conditions [5]. Androgenesis is a method that for the development of haploid and dihaploid plants using anthers [6].

Anther culture technique is utilized for the production of homozygous lines for hybrid seed production. Anther cultures are mainly conducted on Solanaceae and Cruciferae families. But haploid producing ability in the family Solanaceae is rather low [7]. Therefore, it is important to improve anther culture technology for Capsicum.

Anther culture technology for breeding is important to shorten the breeding cycle. Haploid plants obtained from anther cultures are sterile. These plants have only one set of chromosomes and by doubling up their number, plants are made fertile. The resulting plants will be isogenic diploid or homozygous diploid. These homozygous diploid plants show meiotic segregation. The fertile homozygous diploid plants are significant than the sterile haploid plants. Therefore, they can use as pure lines [8].

To develop an effective method for anther culture is essential to determine callus induction media as well as regeneration nutrient media [9]. The objective of the present work is to study the callus induction and further development of callus in different media compositions by using selected *Capsicum annuum* L. varieties.

MATERIALS AND METHODS

The experiment was conducted in the Tissue Culture Laboratory of Horticultural Crops Research and Development Institute (HORDI), Gannoruwa, Peradeniya, Sri Lanka.

The donor plants were grown in the greenhouse of the Horticultural Crops Research and Development Institute. Six hybrid varieties of *Capsicum annuum* L. (HORDI CAH-43, HORDI CAH-44, HORDI CAH-45, HORDI CAH-46, HORDI CAH-47, HORDI CAH-48) and one inbred line (1782) were used in this study.

Unopened flower buds were collected from the mother plants between 8-10 am in the morning. Pre-surface sterilization of anthers was done before taking flower buds to the culture area by dipping them in 70% alcohol for around 30 seconds. Then the surface sterilization of the flower buds was done by using 8% Clorox (commercial detergent) with agitation in the laminar airflow cabinet under the aseptic condition to prevent contamination.

MS medium was used for following two experiments with commercial sugar (30 gL^{-1}) and Myo-inositol (0.1 gL^{-1}) as the osmotic agent.

Experiment 01: Development of an effective medium for callus induction using anthers

As shown in the Table 1, two different callus induction media were tested for the experiment 1. After the sterilization procedure, anthers were exercised using a forcep and those excised anthers were inoculated onto the callus induction medium. For each variety eight treatments were prepared and each treatment consisted of 8 to 12 anthers. Initially, the cultures were kept in the dark condition under 25 ^{0}C and with the commencement of the formation of calli, the cultures were kept under the light of 2000 lux for 16 hrs photoperiod.

Table-1: Callus induction media

Callus induction (CI) medium	Composition of the medium
CI-1	MS + 30 gL^{-1} Sucrose + 0.1gL^{-1} Inositol + 6 gL^{-1} Agar + 0.1gL^{-1} BAP + 5 mgL^{-1} NAA
CI-2	MS + 30 gL^{-1} Sucrose + 0.1 gL^{-1} Inositol + 6 gL^{-1} Agar + 0.1 gL^{-1} BAP + 2.5 mgL^{-1} NAA

Experiment 02: Find out a suitable medium for further development of anther derived capsicum callus

As shown in the Table 2, six different treatments were tested for experiment 2. After the formation of calli in the experiment 1, those were transferred to the media as shown in Table 2 for further development. Then the cultures were placed under the light of 2000 lux for 16 hrs photoperiod at 25 ^{0}C of temperature.

Table -2: Treatments for further development of callus

Treatment	Composition
M-1	MS + 30 gL^{-1} Sucrose + 0.1 gL^{-1} Inositol + 0.1 gL^{-1} BAP + 6 gL^{-1} Agar + 1.5 mgL^{-1} Kinetin
M-2	MS + 30 gL^{-1} Sucrose + 0.1 gL^{-1} Inositol + 0.1 gL^{-1} BAP + 6 gL^{-1} Agar + 2.0 mgL^{-1} Kinetin
M-3	MS + 30 gL^{-1} Sucrose + 0.1 gL^{-1} Inositol + 0.1 gL^{-1} BAP + 6 gL^{-1} Agar + 2.5 mgL^{-1} Kinetin
M-4	MS + 30 gL^{-1} Sucrose + 0.1 gL^{-1} Inositol + 0.1 gL^{-1} BAP + 6 gL^{-1} Agar + 1.5 mgL^{-1} Glycine
M-5	MS + 30 gL^{-1} Sucrose + 0.1 gL^{-1} Inositol + 0.1 gL^{-1} BAP + 6 gL^{-1} Agar + 2.0 mgL^{-1} Glycine
M-6	MS + 30 gL^{-1} Sucrose + 0.1 gL^{-1} Inositol + 0.1 gL^{-1} BAP + 6 gL^{-1} Agar + 2.5 mgL^{-1} Glycine

DATA COLLECTION

Following parameters were recorded in regular intervals.

- **Callus formation percentage**
- **Callus greening percentage**
- **Callus enlargement percentage**

Experimental design and data analysis

The experiment was arranged as a Completely Randomized Design (CRD). The treatment effect was statistically analyzed by using Analysis of Variance (ANOVA) and mean separation was done by Duncan's Multiple Range Test (DMRT). R statistical software was used for statistical analysis.

RESULTS AND DISCUSSION

Experiment 01: Development an effective medium for callus induction using anthers

Formation of callus, initiated within 14 days after culturing. There was no significant difference between 5 mgL^{-1} NAA (CI-1) and 2.5 mgL^{-1} (CI- 2) for callus induction. However, the highest callus percentage (52.17%) was observed in media supplemented with 2.5 mgL^{-1} NAA concentration (Table 3).

Table- 3: Callus formation percentage in different concentrations of NAA

llus induction medium	Concentration of NAA (mgL^{-1})	formation (%)
CI-1	5.0	44.93^{a}
CI-2	2.5	52.17^{a}

Bandara, D. C., Samarajeewa, P. K. *et al*. [10] reported that anther callus formation frequency is higher when medium supplemented with low concentrations of NAA and it reduced significantly at high levels of NAA. As similar to this study, [11] reported that in 4 mgL^{-1} NAA, no callus formation was obtained and medium containing 2 mgL^{-1} NAA was given better callus from Capsicum anthers.

Since 2.5 mgL^{-1} NAA (CI-2) given better callus formation than 5.0 mgL^{-1} NAA (CI-1), 2.5 mgL^{-1} NAA (CI-2) was selected for further studies.

Variety wise comparison in 2.5 mgL^{-1} NAA (CI-2)

In this study variety wise comparison had a significant ($p<0.05$) effect of anther callus formation in 2.5 mgL^{-1} NAA (Table 4). According to Duncan's Multiple Range Test there was a considerable significant difference among HORDI CAH-45 and other varieties. The highest callus formation percentage (59.32%) was observed in HORDI CAH-45 (Figure 1) and followed by HORDI CAH-46 (55.71%) and HORDI CAH-47 (50.19%). The lowest callus formation percentage (5.49%) was obtained from inbred line (1782). In this study, all hybrid varieties were given better performances than the inbred variety.

Table-4: Variety-wise callus formation percentage in 2.5 mgL^{-1} NAA concentration

Variety	Mean value of callus formation (%)
HORDI CAH 43	29.04^{cd}
HORDI CAH 44	40.77^{bc}
HORDI CAH 45	59.32^{a}
HORDI CAH 46	55.71^{ab}
HORDI CAH 47	50.19^{ab}
HORDI CAH 48	19.71^{de}
1782	5.49^{e}

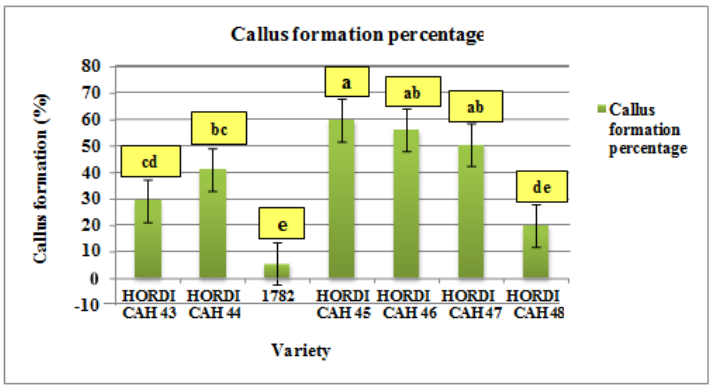

Fig-1: Callus formation percentage in 2.5 mgL^{-1} NAA

Birsin, M. A *et al*. [12] reported that the highest callus percentage (13.71%) of Capsicum anthers were responded in F1 hybrid (No.1647 x No.1969). According to [9] in comparison of the reaction of the inbred Capsicum lines and hybrids, the highest frequency of callus formation was obtained in F1 hybrids due to its hybrid vigour. Indicated that 15 hybrids were obtained positive callus induction between 90-100% according to his study [12].

In this experiment, varieties that given highest callus induction percentages (HORDI CAH- 43, HORDI CAH-44, HORDI CAH-45, HORDI CAH-46, HORDI CAH-47) were used to study further development of anther derived Capsicum callus.

Experiment 02: Find out a suitable medium for further development of anther derived Capsicum callus

Callus enlargement

Callus enlargement had a significant ($p<0.05$) effect among media contained with Glycine and Kinetin (Table 5). According to Duncan's Multiple Range Test, three different concentrations of Kinetin media (M-1, M-2, and M-3) showed similar grouping (b) and three different concentrations of Glycine media (M-4, M-5, and M-6) showed similar grouping (a). However, mean value of callus enlargement percentage was higher in three different concentrations (1.5, 2.0 and 2.5 mgL^{-1}) of Glycine than Kinetin media (Figure 2). Callus enlargement in 2 mgL^{-1} Kinetin and 2 mgL^{-1} Glycine is shown in Plate 1.

Table -5: Mean value of callus enlargement percentage

	Treatment	Mean value of callus enlargement (%)
M-1	1.5 mgL^{-1} Kinetin	50.81^{b}
M-2	2.0 mgL^{-1} Kinetin	50.89^{b}
M-3	2.5 mgL^{-1} Kinetin	53.16^{b}
M-4	1.5 mgL^{-1} Glycine	91.58^{a}
M-5	2.0 mgL^{-1} Glycine	84.28^{a}
M-6	2.5 mgL^{-1} Glycine	80.38^{a}

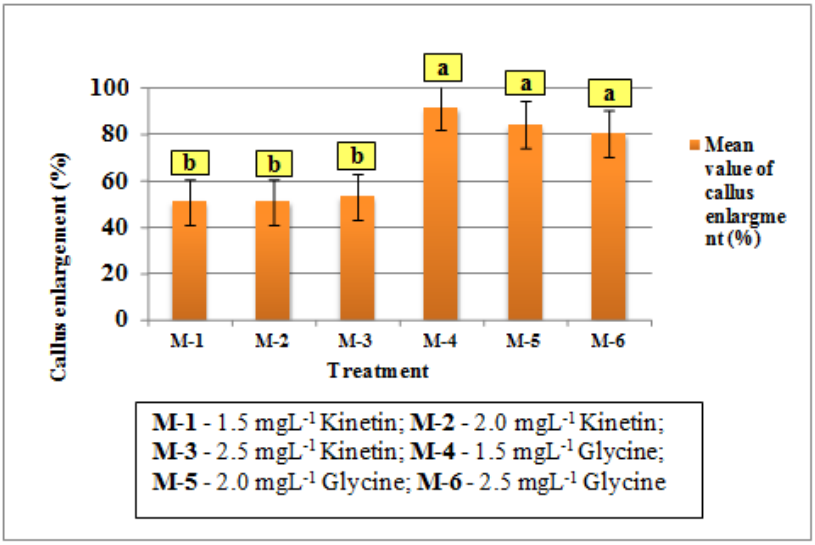

Fig-2: Mean values of callus enlargement percentage in different concentrations of Kinetin and Glycine media

Ashok Kumar, H. G., & Murthy, H. N [13] reported that 2 mM Glycine enhanced embryo induction in cultured anthers of Cucumber. And also reported that in the absence of Glycine like amino acids possessed a low percentage of embryogenic callus production in Cucumber.

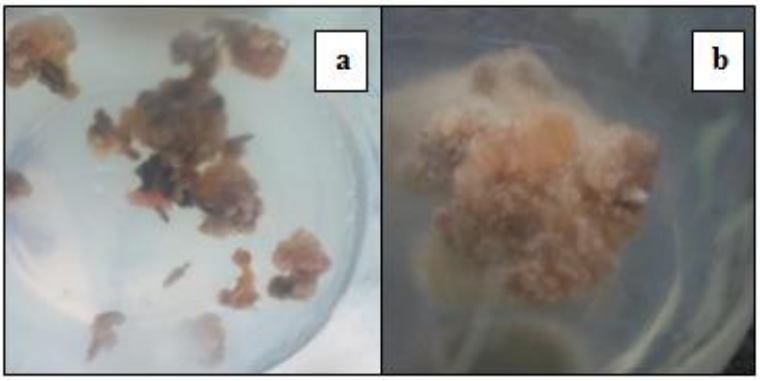

Plate-1: Callus enlargement (a) Anther derived Capsicum callus formation in 2 mgL^{-1} Kinetin from HORDI CAH-47 (b) Anther derived Capsicum callus formation in 2 mgL^{-1} Glycine from HORDI CAH-43

Table-6: Callus enlargement percentage amoung different hybrid varieties

	M-1	M-2	M-3	M-4	M-5	M-6
	1.5 mgL^{-1} Kinetin	2.0 mgL^{-1} Kinetin	2.5 mgL^{-1} Kinetin	1.5 mgL^{-1} Glycine	2.0 mgL^{-1} Glycine	2.5 mgL^{-1} Glycine
HORDI CAH-43	43.84	50.53	45.64	98.38	99.25	97.38
HORDI CAH-44	49.92	43.17	50.84	83.09	63.46	67.39
HORDI CAH-45	57.87	61.62	52.40	91.71	93.21	72.72
HORDI CAH-46	44.70	58.93	52.10	85.94	70.97	68.19
HORDI CAH-47	57.74	40.21	64.85	98.80	94.56	96.23

The callus enlargement percentage had a significant ($p<0.05$) effect among different hybrid varieties. The highest percentage of callus enlargement (99.25%) was obtained in 2.0 mgL^{-1} Glycine (Plate 1b) from HORDI CAH-43 (Table 6) and (Figure 3). The lowest percentage of callus enlargement (40.21%) was obtained in 2.0 mgL^{-1} Kinetin (Plate 1a) from HORDI CAH-47.

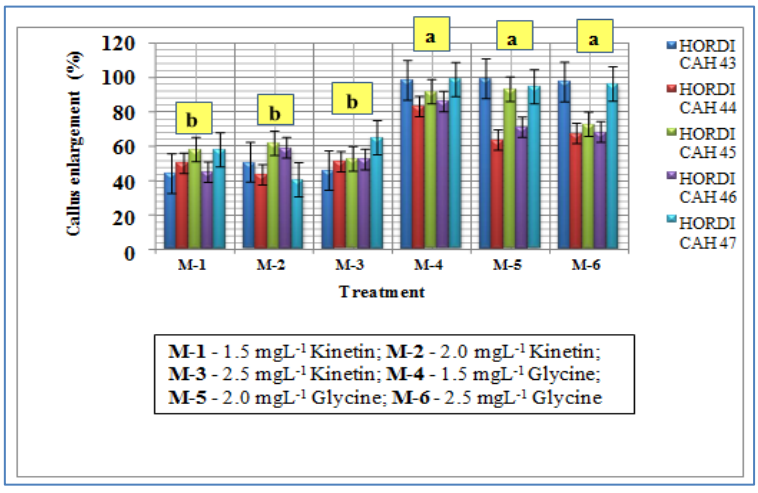

Fig-3: Callus enlargement percentage in different hybrid varieties

Callus greening

In this experiment, some of the callus turned green after 2 to 8 weeks of culturing. With the influence of light, the crystalline calli were turned into soft pale green colour (Plate 2a). Then the middle top region of the callus became white colour. Subsequently, the whole callus gradually turned green colour (Plate 2b).

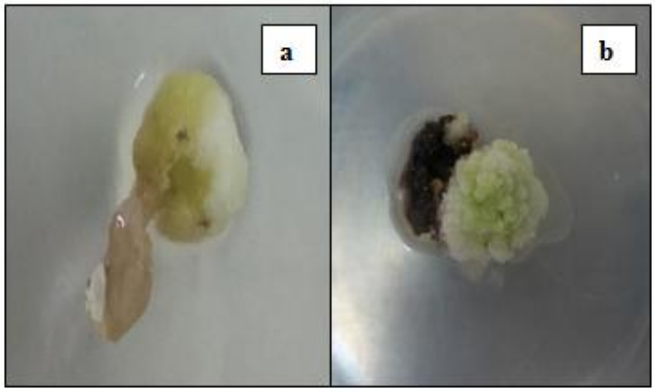

Plate-2: Greening of callus (a) Soft pale green colour Capsicum anther callus (b) Compacted green callus

Table-7: Mean value of callus greening percentage

	Treatment	Mean value of callus greening (%)
M-1	1.5 mgL^{-1} Kinetin	35.56^{ab}
M-2	2.0 mgL^{-1} Kinetin	51.11^{a}
M-3	2.5 mgL^{-1} Kinetin	22.22^{b}
M-4	1.5 mgL^{-1} Glycine	0
M-5	2.0 mgL^{-1} Glycine	0
M-6	2.5 mgL^{-1} Glycine	0

In this study callus greening had a significant ($p<0.05$) effect. According to Duncan's Multiple Range Test, the highest mean value of callus greening percentage was obtained in 2 mgL^{-1} Kinetin medium (Table 7). No greening was expressed in the media supplemented with Glycine.

[14] reported that 2 mgL^{-1} of Kinetin was required to induce chlorophyll synthesis of callus. Similarly, [15] reported that 2 mgL^{-1} Kinetin has an effect on the greening of calli which has a tendency on regeneration [16]. Revealed that the addition of 2 mgL^{-1} of Kinetin resulted in colours of callus from pale green and yellowish green to green. And he reported low or absence of Kinetin results no callus greening in his study due to chlorophyll degradation.

Table-8: Callus greening percentage in different hybrid varieties

	M-1	M-2	M-3	M-4	M-5	M-6
	1.5 mgL^{-1} Kinetin	2.0 mgL^{-1} Kinetin	2.5 mgL^{-1} Kinetin	1.5 mgL^{-1} Glycine	2.0 mgL^{-1} Glycine	2.5 mgL^{-1} Glycine
HORDI CAH-43	66.67	66.67	33.33	0	0	0
HORDI CAH-44	22.22	22.22	0	0	0	0
HORDI CAH-45	44.44	88.89	44.45	0	0	0
HORDI CAH-46	33.33	44.45	22.22	0	0	0
HORDI CAH-47	11.11	33.33	11.11	0	0	0

In this study callus greening percentage had a significant ($p<0.05$) effect. According to the Table 8, the highest percentage of callus greening (88.89%) was obtained in 2.0 mgL^{-1} Kinetin from HORDI CAH-45 (Figure 4).

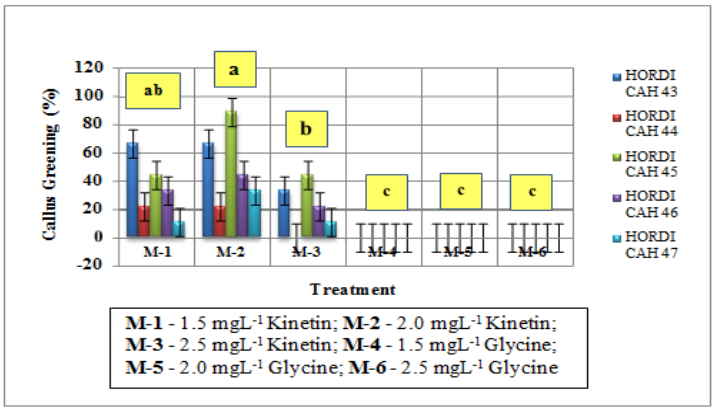

Fig-4: Callus greening percentage in different hybrid varieties

CONCLUSION

The experiment revealed that MS medium supplemented with 2.5mgL^{-1} NAA is the most suitable for callus initiation. HORDI CAH-45 F1 hybrid variety was given the highest percentage of callus formation (59.32%) in 2.5 mgL^{-1} NAA. The best percentage of callus enlargement (99.25%) was obtained in 2.0 mgL^{-1} Glycine from HORDI CAH-43. The best percentage of callus greening (88.89%) was obtained in 2.0 mgL^{-1} Kinetin from HORDI CAH-45. Regeneration ability has to be studied further with different combinations of Kinetin and Glycine to induce shoot and root from green callus.

REFERENCES

1. Zhigila, D. A., Abdulrahaman, A. A., Kolawole, O. S., & Oladele, F. A. (2014). Fruit morphology as taxonomic features in five varieties of capsicum annuum L. solanaceae. *Journal of Botany*.
2. Sánchez, M. A., Coronado, Y. M., & Coronado, A. C. M. (2020). Androgenic studies in the production of haploids and doubled haploids in capsicum spp. *Revista Facultad Nacional de Agronomia Medellin*, *73*(1), 9047–9056.
3. Jaramillo, J. (1988). *Anther culture in the tomato (Lycopersicon esculentum Mill) callus and plantlet production studies*. 225.
4. Pauk, J., Mihaly, R., Monostori, T., Puolimatka, M. (2003). Protocol of triticale (x Triticosecale Wittmack) microspore culture. In: Maluszynski, M., Kasha, K. J., Forster, B. P., Szarejko, I. (eds). Doubled haploid production in crop plants, a mannual. Kluwer Academic Publishers, Dordrecht, 129–134.
5. Nowaczyk, P., & Kisiała, A. (2006). Effect of selected factors on the effectiveness of Capsicum annuum L. anther culture. *Journal of Applied Genetics*, *47*(2), 113– 117.
6. Dumas de Vaulx, R., Chambonnet, D., Pochard, E. (1981). Culture in vitro d'anthéres de piment (*Capsicum annuum* L.) amélioration des taux d'obtention de plantes chez différents génotypes par des traitements. Agronomy 1, 859–864.
7. Matsubara, S., Yamamoto, M., Hyun Jo, M., & Murakami, K. (1998). Embryoid and Callus ormation from Microspores by Anther Culture from July to November in Pepper (Capsicum annuum L.). In Scientific Reports of the Faculty of Agriculture (Vol. 87, pp. 117–122).
8. Biostructures, H. (2020). Advantages of Pollen Culture over Anther Culture (With Diagram). 1–18.
9. Rodeva, V. N., Irikova, T. P., & Todorova, V. J. (2004). Anther culture of pepper (capsicum annuum l.): Comparative study on effect of the genotype. *Biotechnology and Biotechnological Equipment*, *18*(3), 34–38.
10. Bandara, D. C., Samarajeewa, P. K., & Lanka, S. (2008). The Effect of Plant Growth Regulators on Anther Culture Response and Plant Regeneration in Selected Sri Lankan Indica Rice Varieties, Japonica Varieties and Their Inter- Sub Specific Hybrids. 20, 243–250.
11. Comlekcioglu, N., Büyükalaca, S., & Abak, K. (2001). Effect of silver nitrate on haploid embryo induction by anther culture in pepper (Capsicum annuum L.), XIth EUCARPIA Meeting on Genetics and Breeding of Capsicum & Eggplant. Antalya, Turkey, 133-136.
12. Birsin, M. A. (2005). The effect of hybrid vigor on callus induction and plant regeneration from mature embryo culture of barley (Hordeum vulgare). 67–74.
13. Ashok Kumar, H. G., & Murthy, H. N. (2004). Effect of sugars and amino acids on androgenesis of Cucumis sativus. Plant Cell, Tissue and Organ Culture, *78*(3), 201–208.
14. Kaul, K., & Sabharwal, P. S. (1971). Effects of Sucrose and Kinetin on Growth and Chlorophyll Synthesis in Tobacco Tissue Cultures. Plant Physiology, *47*(5), 691–695.
15. Bobkov, S. (2014). Obtaining Calli and regenerated plants in anther cultures of pea. *Czech Journal of Genetics and Plant Breeding*, *50* (2), 123–129.
16. Sari, Y. P., Kusumawati, E., Saleh, C., Kustiawan, W., & Sukartingsih, S. (2018). Effect of sucrose and plant growth regulators on callogenesis and preliminary secondary metabolic of different explant Myrmecodia tuberosa. *Nusantara Bioscience*, *10*(3), 183–192.