Biological Forum – An International Journal **14(1): 890-894(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of 6-Benzylaminopurine (BAP) and Kinetin (Kn) on Callus Induction under *In vitro* Culture of *Aloe vera*

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(Received 05 November 2021, Accepted 06 January, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The BAP (6-benzylaminopurine) and Kn (Kinetin) are important cytokinins, commonly utilizes in plant tissue culture for micropropagation of various plant species. However, their regeneration response under *in vitro* condition highly dependent on the culture condition especially concentration of growth regulators. In the present investigation, effects of different levels of BAP and Kn (0.5 to 6.0 mg/l) were evaluated for callus induction in lateral shoot explant of *Aloe vera.* Differential concentrations of these growth regulators had shown variable effects on callus proliferation in MS (Murashige and Skoog) medium. In BAP incorporated medium, maximum callus induction (0.92 g) was obtained at the concentration of 5.0 mg/l BAP, which was characterized with yellow colour and semi compact texture. However, highest callus weight induced in Kn fortified medium was 1.25 g obtained at 4.0 mg/l. Induced callus morphologically characterizes with whitish yellow colour and friable textured. Among these two growth regulators, Kn was found more efficient than BAP for callus proliferation with respect to days taken for callus initiation and induced callus weight. The results of current investigation give an idea of efficacy of BAP and Kn for morphogenetic effects on callus proliferation. Thus, for best economical use, the current study may help in identification of suitable growth regulator with appropriate level for getting maximum callus induction response of *Aloe vera* under *in-vitro* condition.

Keywords: Aloe, BAP, Callus, Cytokinin, Kinetin, Micropropagation.

INTRODUCTION

Aloe vera (L.) Burm. f. (Carter *et al*., 2011) is a short stemmed, xerophytic, evergreen perennial shrub, grows up to height of 80-100 cm. Flowers are drooping produced in an inflorescence of 90-110 cm tall. Each flower is pendulous, with yellow tubular corolla length of 2-3 cm, hermaphrodite and possess male sterility (Saran *et al*. 2019). It is a desert medicinal plant, immensely utilizes in the pharmaceutical, cosmetic and food industries. It is synonymously known as *Aloe barbadensis* and commonly called "Gwarpatha" or "Ghrit kumari" in Sanskrit (Raksha *et al.,* 2014; Ahmad *et al.,* 2020). Bioactive ingredients of *Aloe* gel known for, anti-inflammatory, anti-tumor, anti-ulcer, anti-cancer, anti-bacterial and anti-viral properties (Jayakrishna *et al.,* 2011). Traditionally, lateral shoots or suckers are used as planting material for cultivation of *Aloe vera* which is a slow and tedious method for its propagation (Bhandari *et al.* 2010). Due to insufficient availability of naturally developed shoots, large scale production of superior quality planting material of *Aloe* is only possible through *in vitro* micropropagation technique.

Callus is the mass of unorganized dividing cells usually induced by varied concentrations of growth hormones in the culture medium. Callus can be produced from a single differentiated cell and many callus cells are totipotent, being able to regenerate the whole plant body. Callus induction and organogenesis in the form of shoot/root/embryogenesis from somatic cells and tissues may occur depending upon the concentration of plant growth regulators such as auxins and cytokinins singly or in combination. Auxins, cytokinins, and auxin-cytokinin interactions are usually considered to be the most important for regulating growth of plant under *in vitro* condition, as these two classes of hormones generally required for root and shoot regeneration (Choudhary *et al.,* 2011). Two major properties of cytokinins that are useful in culture are stimulation of cell division and release of lateral bud dormancy. Cell division is regulated by the joint action of auxins and cytokinins, each of which influences different phases of the cell cycle. Auxins affect DNA replication, whereas cytokinins seem to exert some control over the events leading to mitosis and cytokinesis (Ahmad *et al.,* 2020). Thus plant growth regulators levels in cultures need to be carefully balanced and controlled. The BAP (6-benzylaminopurine) and Kn (Kinetin) are one of the important cytokinins, commonly utilize in plant tissue

culture for micropropagation of various species. Each type of cytokinin has a wide range of physiological effects in different plants. These effects are determined by its concentration, the presence or absence of other growth regulators, genetic makeup and the physiological status of the donor plant and kind of explants. The same physiological response in different tissues even of the same plant may require different growth regulator(s) or different combinations of growth regulators. Therefore, the present investigation was carried out to evaluate the effect of BAP and Kn on callus induction under *in vitro* condition.

MATERIALS AND METHODS

The present investigation was carried out at Sri Karan Narendra Agriculture University Jobner, India at Tissue Culture Laboratory under the department of Plant Breeding and Genetics, during the year 2020-2021. Lateral shoot explant of Jobner Aloe-1 genotype was used for inoculation in MS medium (Murashige and Skoog, 1962). Sizes of 3-4 cm length of lateral shoot explants were used for testing the effects on callus induction of BAP (6-benzylaminopurine) and Kn (Kinetin). Twelve discrete concentrations/levels of BAP and Kn (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 5.5 and 6.0) were incorporated in the medium and responses over callus induction along with control (MS medium devoid of plant growth regulators) were observed. The experiment was laid out in Completely Randomized Design (CRD) using ten replication of each treatment. Inoculated vessels kept in culture room which was maintained at standardized temperature (25 ± 2°C) with light intensity of 3000 lux for 14 hours followed by 10 hours dark in a day.

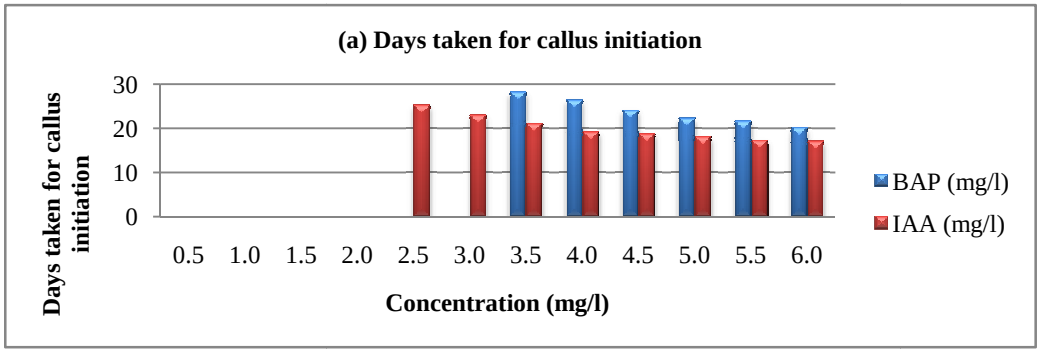
Cultures were observed periodically throughout the experiments and data on days to initiation for callus, callus induction frequency, callus weight, callus colour and texture were recorded after 8 weeks of inoculation. Callus weight was measured in gram using electric balance while data on callus colour and texture were observed on visual basis. Morphogenetic response of explant (per cent) for induction of callus was calculated as:

$$\text{Morphogenetic response (\%)} = \frac{\text{Number of explants response}}{\text{Total number of inoculated explants}} \times 100$$

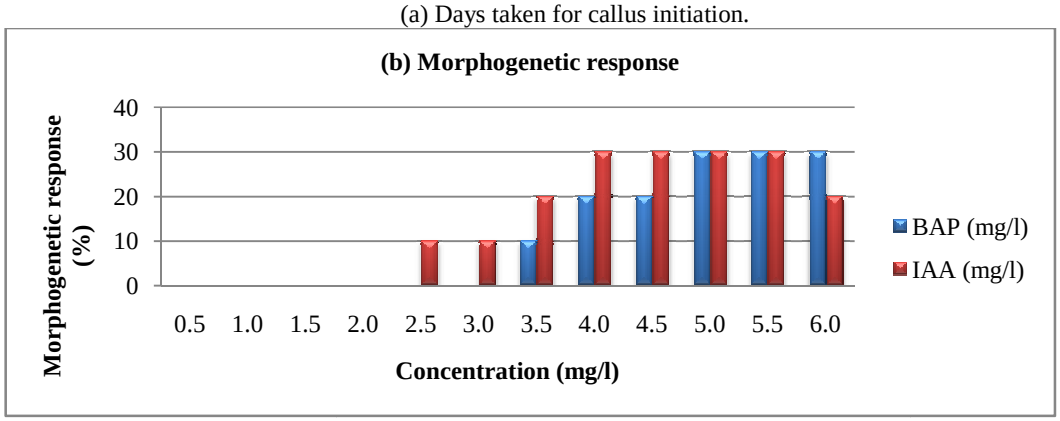
Data were analyzed by using XLSTAT software for means and standard error accordingly as described by Snedecor and Cochran (1972). Test of significance for treatment comparisons were done following Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984). Analysed data depicted in the table as Mean ± Standard Error (SE). Values followed by different letters (as per DMRT) in each column significantly differ at probability of 5 per cent.

RESULTS AND DISCUSSIONS

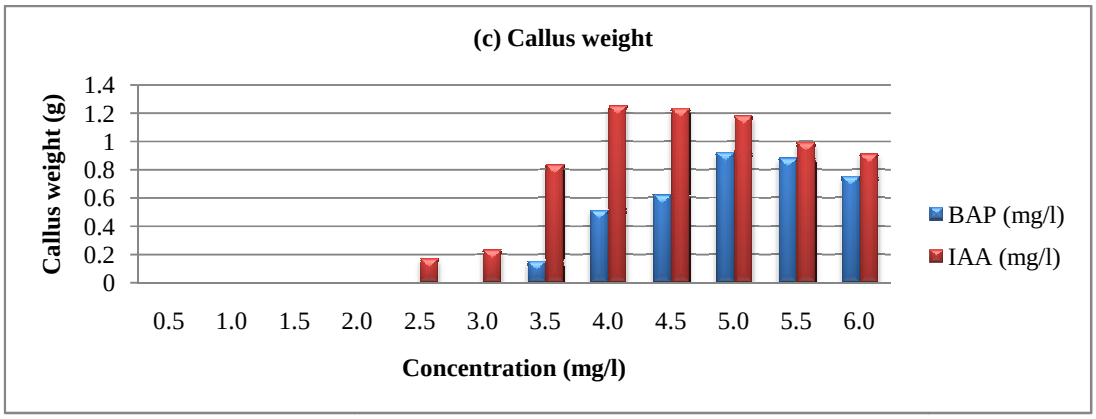
In the present investigation, effect of cytokinins (BAP and Kn) were tested at 0.5 – 6.0 mg/l concentrations for callus induction in lateral shoot explant of *Aloe vera*. Differential levels of BAP and Kn revealed varied effects on callus induction in MS medium. Only higher levels of both cytokinins showed responses with low frequencies of callus weight. A comparative trend of responses of different concentrations of BAP and Kn for days taken for callus initiation, morphogenetic response (%) and callus weight (g) are presented in the Fig. 1.



(a) Days taken for callus initiation.



(b) Morphogenetic response.



(c) Callus weight

Fig. 1. Comparative trends of responses for different concentrations of BAP and Kn used for callus induction in lateral shoot explant of *Aloe vera.*

Callus initiation was not observed up to 3.0 mg/l BAP but slight to medium callus was induced on higher levels with 10 – 30 per cent frequency in 20.20 – 28.20 days (Table 1). Days taken in callus initiation were reduced with increased levels of BAP. Callus weight ranged between 0.15–0.92 g, while maximum callus (0.92 g) was obtained at 5.0 mg/l BAP with 30 per cent frequency and 22.30 days for callus initiation (Fig. 2). Significant differences were seen for callus weight and days taken in callus initiation at 5.0 mg/l BAP with all the levels except 5.5 mg/l BAP. Callus was yellow or pale yellow in colour, characterized with friable, semi compact and loose texture. These observations were in close agreement with the findings of Saggoo and Kaur, (2010), Kumar *et al.* (2017) and Wahab *et al.* (2020).

Table 1: Effect of BAP on callus induction supplemented in the MS medium.

BAP (mg/l)	Days taken for callus initiation	Morphogenetic response (%)	Callus weight (g)	Callus colour	Callus texture
0.5	-	-	-	-	-
1.0	-	-	-	-	-
1.5	-	-	-	-	-
2.0	-	-	-	-	-
2.5	-	-	-	-	-
3.0	-	-	-	-	-
3.5	28.20±0.84[a]	10	0.15±0.01[e]	Yellow	Friable
4.0	26.40±0.43[b]	20	0.51±0.03[d]	Yellow	Semi compact
4.5	24.10±0.23[c]	20	0.62±0.06[c]	Yellow	Semi compact
5.0	22.30±0.21[d]	30	0.92±0.03[a]	Yellow	Semi compact
5.5	21.60±0.16[d]	30	0.88±0.04[a]	Pale yellow	Loose
6.0	20.20±0.29[e]	30	0.75±0.02[b]	Pale yellow	Loose

Values in columns represent Mean ± SE; Values followed by different letters in each column are significantly different ($p<0.05$); (-) = No response

In case of Kn mediated culture medium, callus initiation was observed at 2.5 mg/l to 6.0 mg/l Kn with 10 – 30 per cent frequency in 17.10 – 25.20 days of initiation. Days taken for callus initiation were declined with enhanced levels of Kn; however, significant differences were present at all the levels of treatments. Callus induction was increased with increasing levels of Kn, reached maximum (1.25 g) at 4.0 mg/l and declined progressively on higher concentration (Table 2 and Fig. 3). Callus weight induced at 4.0 mg/l Kn was significantly higher than other levels except 4.5 and 5.0 mg/l. Callus was characterized with whitish yellow to yellow brown colour and friable, semi compact and loose textured. Similar observation was obtained by Saggoo and Kaur (2010) in *Aloe.*

Kinetin was found more efficient than BAP with respect to days taken for callus initiation, callus induction frequency and for callus weight (Fig. 1). Callus induced in both, BAP and Kn mediated culture media were low in amount and also seems to be poor in regeneration response. However, profuse amount of callus had been induced by using different type of auxins (2,4-D, NAA, IAA) either alone or in combinations with cytokinins in different genotypes of *Aloe* (Saggo and Kaur, 2010; Rathore *et al.*, 2011; Kim *et al.*, 2012; Badar *et al.*, 2013; Kumari and Naseem, 2015; Jakhar *et al.*, 2020). Therefore it is concluded that both these growth regulators (BAP and Kn) are less efficient for callus induction as compare to auxins, hence for higher efficacy and economical use they can be utilize in combination with auxins.

Fig. 2. Callus induction in lateral shoot explant in the MS medium supplemented with BAP at 5.0 mg/l.

Fig. 3. Callus induction in lateral shoot explant in the MS medium supplemented with Kn at 4.0 mg/l.

Table 2: Effect of Kn on callus induction supplemented in the MS medium.

Kn (mg/l)	Days taken for callus initiation	Morphogenetic response (%)	Callus weight (g)	Callus colour	Callus texture
0.5	-	-	-	-	-
1.0	-	-	-	-	-
1.5	-	-	-	-	-
2.0	-	-	-	-	-
2.5	25.20±0.36[a]	10	0.17±0.01[d]	Whitish yellow	Friable
3.0	23.00±0.26[b]	10	0.23±0.01[d]	Whitish yellow	Friable
3.5	21.10±0.28[c]	20	0.83±0.04[c]	Whitish yellow	Friable
4.0	19.20±0.29[d]	30	1.25±0.05[a]	Whitish yellow	Friable
4.5	18.80±0.25[d]	30	1.23±0.06[a]	Whitish yellow	Semi compact
5.0	18.10±0.38[e]	30	1.18±0.05[a]	Whitish yellow	Semi compact
5.5	17.30±0.30[f]	30	0.99±0.06[b]	Yellow brown	Loose
6.0	17.10±0.23[f]	20	0.91±0.05[bc]	Yellow brown	Loose

Values in columns represent Mean ± SE; Values followed by different letters in each column are significantly different ($p<0.05$); (-) = No response

CONCLUSION

In the present investigation, effects of different levels of BAP and Kn were evaluated for callus induction in lateral shoot explant of *Aloe vera.* In BAP incorporated medium, maximum callus induction (0.92 g) was obtained at the concentration of 5.0 mg/l, while highest callus weight induced in Kn fortified medium was 1.25 g at 4.0 mg/l. Consequently, Kn found more proficient than BAP for callus proliferation in lateral shoot explant. The results of current investigation give an idea of effectiveness of BAP and Kn for morphogenetic effects on callus proliferation. Thus, for best economical use, the current study may help in the identification of suitable growth regulator with appropriate level for getting maximum callus induction response for *in-vitro* micropropagation of *Aloe vera*.

Acknowledgements. The author acknowledges the support from staff of Tissue culture laboratory and Plant Breeding & Genetics department of S. K. N. College of Agriculture, S. K. N. Agriculture University, Jobner, India. The corresponding author is also thankful to the ICAR, New Dehli for awarding ICAR-SRF for Ph.D. degree, which greatly helped financially during course of research work.
Conflict of Interest. None.

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How to cite this article: Sarfraz Ahmad, M.L. Jakhar, D.K. Gothwal, Manohar Ram, B.L. Kumhar and G.L. Kumawat (2022). Effect of 6-Benzylaminopurine (BAP) and Kinetin (Kn) on Callus Induction Under *In vitro* Culture of *Aloe vera*. *Biological Forum – An International Journal*, *14*(1): 890-894.