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Optimization of In Vitro Growth of Mung Bean (*Vigna radiata* L.) Using BAP and GA_3 for Planting Material Production

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Abstract:
Uniform, high-quality planting material of mung bean (*Vigna radiata* L.) is limited. This study evaluated the effects of 6-benzylaminopurine (BAP) and gibberellic acid (GA_3) on the in vitro growth of mung bean. The experiment (October–November 2024, Politeknik Negeri Jember, Indonesia) used a factorial completely randomized design with BAP at five concentrations (2.5, 3.5, 4.5, 5.5, and 6.5 mg L^{-1}) and GA_3 at two concentrations (4 and 5 mg L^{-1}), replicated three times. Six growth variables were analyzed by ANOVA and Duncan's multiple range test. GA_3 concentration had a highly significant effect on germination time: 4 mg L^{-1} GA_3 produced faster germination (6.87 days after planting) than 5 mg L^{-1} (8.13 days). The BAP × GA_3 interaction significantly affected the number of roots, with the highest values in 4.5 mg L^{-1} BAP + 5 mg L^{-1} GA_3 (2.22) and 6.5 mg L^{-1} BAP + 5 mg L^{-1} GA_3 (2.00). Plant height, number of leaves, stem diameter, and dry weight were not affected. A medium containing 4 mg L^{-1} GA_3 is recommended for rapid germination, and 4.5 mg L^{-1} BAP with 5 mg L^{-1} GA_3 for better root formation.

Keywords:
benzylaminopurine, gibberellic acid, in vitro culture, mung bean, plant growth regulator

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INTRODUCTION

Mung bean (*Vigna radiata* L.) is an important food legume in Asia. Its seeds contain about 21–31% easily digestible protein, together with dietary fiber, minerals, and bioactive compounds such as polyphenols and peptides with antioxidant and antidiabetic activities (Hou et al., 2019; Mehta et al., 2021). The crop has a short life cycle, tolerates a range of growing conditions, and fixes atmospheric nitrogen through symbiosis with rhizobia, which improves soil fertility in cropping systems (Dwivedi et al., 2023). Despite these advantages, national mung bean productivity in Indonesia remains relatively low. One of the main reasons is the limited availability of high-quality seed. Many farmers use seeds saved from previous harvests without adequate selection, which leads to uneven growth, low vigor, and low yields. Seed-borne diseases transmitted through such seeds further reduce crop establishment, and the multiplication of new superior varieties through conventional seed production takes several seasons before enough seed reaches farmers.

Mung bean is grown mainly by smallholder farmers in Indonesia, often as a second crop after rice, under rainfed conditions with low inputs. Under such conditions, the quality of the seed used at planting strongly determines crop establishment and yield. Improving the supply of healthy, genetically uniform planting material is therefore one of the simplest ways to raise productivity, and

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biotechnological approaches can help to accelerate the multiplication of improved varieties and conserve local germplasm (Dwivedi et al., 2023).

Plant tissue culture offers a way to produce large numbers of uniform and pathogen-free plants in a short time under controlled conditions. It also supports the conservation of local germplasm, the multiplication of selected genotypes, and genetic improvement programs (Delgado-Paredes et al., 2021). For legumes such as mung bean, in vitro seed germination and seedling culture provide clean starting material for shoot multiplication, regeneration, and transformation, and a rapid, uniform germination stage is therefore the first step of an efficient in vitro propagation system (Rathore et al., 2023; Iqbal et al., 2022). In addition, in vitro culture allows the effects of plant growth regulators on early growth to be studied without the influence of soil and weather.

However, legumes are generally considered difficult to culture in vitro. Their regeneration capacity is often low and strongly genotype-dependent; explants may release phenolic compounds that cause browning, and plantlets may suffer from hyperhydricity and poor acclimatization (Kanishka et al., 2023; Haliloğlu et al., 2023). These problems necessitate optimizing the composition of the culture medium, particularly the types and concentrations of plant growth regulators, for each species and purpose. A well-developed root system is also essential, because plantlets with few roots often fail during transfer to ex vitro conditions (Mohammed et al., 2023).

Cytokinins and gibberellins are two groups of plant growth regulators that are widely used in tissue culture. 6-Benzylaminopurine (BAP) is a synthetic cytokinin that stimulates cell division, promotes shoot initiation, and is the most frequently used cytokinin for the in vitro culture of *Vigna* species (Rathore et al., 2023). Cytokinin signaling is a central regulator of de novo shoot organogenesis (Hnatuszko-Konka et al., 2021), but high cytokinin levels can inhibit root growth and induce physiological disorders such as hyperhydricity (Ersali, 2024). Gibberellic acid (GA_3) promotes seed germination by counteracting abscisic acid and activating hydrolytic enzymes that mobilize seed reserves (Tuan et al., 2018; Vishal and Kumar, 2018). Gibberellins also regulate cell division and elongation in the root meristem (Rizza et al., 2021; Shtin et al., 2022). In vitro, GA_3 has been used to improve seed germination in several species; for example, a medium containing 5.0 mg L^{-1} GA_3 achieved 90% seed germination in *Loxopterygium huasango* (Millones-Yamunaqué et al., 2021).

Cytokinins and gibberellins do not act independently. Their effects depend on their balance with one another and with endogenous hormones such as auxin, and their interactions control the development of both shoots and roots (Velandia et al., 2024; Ma et al., 2024). In mung bean, exogenous cytokinin and GA_3 improved growth, biomass, and photosynthesis under waterlogging stress, with GA_3 acting more on shoot growth and cytokinin more on root development (Islam et al., 2021; Islam et al., 2022). In common bean, GA_3 applied to seeds or plants affected stem height and root dry mass (Jaques et al., 2019). However, information on the combined effects of BAP and GA_3 concentrations on the germination and early in vitro growth of mung bean is still limited, and the optimal combination for producing vigorous plantlets has not been established. Most previous in vitro studies on mung bean used low BAP concentrations (around 0.5–1.0 mg L^{-1}) to induce multiple shoots from cotyledonary nodes, shoot tips, or embryonic axes (Rathore et al., 2023; Iqbal et al., 2022), whereas the responses of whole seedlings to higher BAP concentrations combined with GA_3 have rarely been reported. Such information is needed to design media that support both rapid germination and the formation of vigorous plantlets with adequate roots in a single culture step.

Therefore, this study aimed to determine the effects of BAP concentration, GA_3 concentration, and their interaction on the in vitro germination and growth of mung bean, and to identify the combination that best supports the production of planting material. We hypothesized that GA_3 would

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mainly affect germination, whereas the combination of BAP and GA_3 would affect root and shoot development.

METHOD

The research was conducted from October to November 2024 at the Tissue Culture Laboratory, Politeknik Negeri Jember, Jalan Mastrip, Sumbersari District, Jember Regency, East Java, Indonesia. All aseptic work was carried out in a laminar airflow cabinet. Seeds of mung bean (*Vigna radiata* L.) variety were used as explants. Healthy, uniform seeds of similar size, without visible damage or disease, were selected before sterilization. The seeds were washed under running tap water with a few drops of detergent and rinsed with distilled water. The sterilized seeds were blotted on sterile filter paper before planting.

Stock solutions of BAP and GA_3 were prepared at a concentration of 100 mg L^−1^. BAP was dissolved in a few drops of 1 N NaOH and GA_3 in a few drops of ethanol before being brought to volume with distilled water. The stock solutions were stored in a refrigerator at 4 °C and used within two weeks. The volume of stock solution added to each medium was calculated using the dilution formula $V_1 \times C_1 = V_2 \times C_2$, where V_1 and C_1 are the volume and concentration of the stock solution, and V_2 and C_2 are the volume and concentration of the medium.

The basal medium was Murashige and Skoog (MS) medium, confirmed and supplemented with 30 g L^−1^ sucrose, and solidified. BAP and GA_3 were added from stock solutions according to the treatments (Table 1). The pH of the medium was adjusted to 5.8 before adding agar, and the medium was dispensed into culture bottles and sterilized in an autoclave at 121 °C and 1.1 kg cm^{-2} for 15–20 min. If GA~3~ was filter-sterilized and added after autoclaving, state this here.

The experiment was arranged in a factorial completely randomized design (CRD) with two factors. The first factor was BAP concentration at five levels: B1 = 2.5 mg L^{-1}, B2 = 3.5 mg L^{-1}, B3 = 4.5 mg L^{-1}, B4 = 5.5 mg L^{-1}, and B5 = 6.5 mg L^{-1}. The second factor was GA_3 concentration at two levels: G1 = 4 mg L^{-1} and G2 = 5 mg L^{-1}. The combination of the two factors resulted in 10 treatments (Table 1), each replicated 3 times, yielding 30 experimental units. Each experimental unit consisted of one culture bottle containing three seeds.

Table 1. Treatment combinations of BAP and GA_3 concentrations

BAP concentration	G1 (GA_3 4 mg L^{-1})	G2 (GA_3 5 mg L^{-1})
B1 (2.5 mg L^{-1})	B1G1	B1G2
B2 (3.5 mg L^{-1})	B2G1	B2G2
B3 (4.5 mg L^{-1})	B3G1	B3G2
B4 (5.5 mg L^{-1})	B4G1	B4G2
B5 (6.5 mg L^{-1})	B5G1	B5G2

Note: Each combination was replicated three times (30 experimental units in total).

The data were analyzed according to the linear model of a two-factor CRD: $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$, where Y_{ijk} is the observation for the *i*-th BAP level, *j*-th GA_3 level, and *k*-th replication, μ is the general mean, α_i is the effect of the *i*-th BAP concentration, β_j is the effect of the *j*-th GA_3 concentration, $(\alpha\beta)_{ij}$ is the interaction effect, and ε_{ijk} is the experimental error.

The sterilized seeds were planted on the surface of the medium using sterile forceps, and the culture bottles were closed with plastic caps/aluminium foil and sealed with plastic wrap. The cultures were incubated in the culture room at 25 ± 2 °C under a 16-h photoperiod provided by white fluorescent/LED lamps with a specified light intensity. The cultures were checked daily for

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contamination, and contaminated bottles were removed and recorded. The observation period lasted until 32 days after planting (DAP). Culture bottles were placed on the shelves of the culture room in a completely random arrangement, and their positions were re-randomized weekly to minimize the effect of differences in light intensity among shelf positions.

Six variables were observed (Table 2). Germination time was recorded daily as the number of days from planting to radicle emergence from the seed coat, confirming the germination criterion. Plant height was measured at 32 DAP from the base of the stem to the shoot tip, and the number of leaves was counted on the same day. The number of roots was counted as the number of roots formed per plantlet at the end of the experiment. Plantlet dry weight was determined at the end of the experiment after the plantlets were removed from the medium, washed to remove adhering agar, and dried in an oven at 70 °C for 48 h to constant weight.

Table 2. Observed variables, units, time, and method of observation

Variable	Unit	Time	Method
Germination time	DAP	Daily	Days from planting to radicle emergence
Plant height	cm	32 DAP	From stem base to shoot tip, ruler
Number of leaves	leaves	32 DAP	Counted per plantlet
Number of roots	roots	End of culture	Counted per plantlet
Stem diameter	mm	End of culture	Digital caliper
Plantlet dry weight	g	End of culture	Oven-dried to constant weight

All measurements were taken on every plantlet in each experimental unit, and the mean of the plantlets in a unit was used as the value for that replication in the statistical analysis. Plantlets lost to contamination were excluded, and the number of plantlets observed per unit was recorded. To avoid bias, measurements were taken by the same person using the same instruments throughout the experiment.

The data were analyzed using analysis of variance (ANOVA) for a two-factor CRD to determine the effects of BAP, GA_3, and their interaction. Effects were declared significant at the 5% level and highly significant at the 1% level. When the ANOVA showed a significant effect, the means were compared using Duncan's multiple range test (DMRT) at the same probability level. For the significant interaction, the ten treatment means were compared, and the marginal means for each factor were calculated from the treatment means to describe the main trends. Data were analyzed using software programmed on a computer.

RESULTS

The ANOVA showed that mung bean responses to BAP and GA_3 differed across the observed variables (Table 3). GA_3 concentration had a highly significant effect on germination time, whereas BAP concentration and the BAP × GA_3 interaction did not. The number of roots was significantly affected by BAP concentration and by the interaction between BAP and GA_3. Plant height and the number of leaves at 32 DAP, stem diameter, and plantlet dry weight were not significantly affected by either factor or their interaction.

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Table 3. Summary of analysis of variance for the effects of BAP and GA_3 on the in vitro growth of mung bean

Variable	BAP (B)	GA_3 (G)	B × G
Germination time	ns	**	ns
Plant height (32 DAP)	ns	ns	ns
Number of leaves (32 DAP)	ns	ns	ns
Number of roots	*	ns	*
Stem diameter	ns	ns	ns
Plantlet dry weight	ns	ns	ns

Note: ** = highly significant ($p < 0.01$); * = significant ($p < 0.05$); ns = not significant ($p \geq 0.05$).

Mung bean seeds germinated within about one to two weeks after planting. Seeds cultured on medium containing 4 mg L^{-1} GA_3 germinated at 6.87 DAP on average, which was significantly faster than those on medium containing 5 mg L^{-1} GA_3 (8.13 DAP) according to DMRT at the 1% level (Table 4; Figure 1a). Increasing the GA_3 concentration from 4 to 5 mg L^{-1} therefore delayed germination by 1.26 days (18.3%). In other words, germination on the medium with 4 mg L^{-1} GA_3 was 15.5% faster than on the medium with 5 mg L^{-1} GA_3.

Table 4. Effect of GA_3 concentration on the germination time of mung bean in vitro

GA_3 concentration	Germination time (DAP)	Relative to G2 (%)
G1 (4 mg L^{-1})	6.87 a	−15.5
G2 (5 mg L^{-1})	8.13 b	–

Note: Means followed by different letters are significantly different according to DMRT at the 1% level (DMRT value = 1.004). Values are means over the five BAP concentrations.

The number of roots per plantlet ranged from 0.89 to 2.22 (Table 5; Figure 1b). The highest values were obtained with 4.5 mg L^{-1} BAP + 5 mg L^{-1} GA_3 (B3G2; 2.22 roots) and 6.5 mg L^{-1} BAP + 5 mg L^{-1} GA_3 (B5G2; 2.00 roots), which were significantly higher than all other combinations. The remaining eight combinations produced 0.89–1.22 roots and did not differ significantly from each other.

The interaction pattern shows that the effect of GA_3 on rooting depended on the BAP concentration. At 4 mg L^{-1} GA_3, the number of roots was almost constant (0.89–1.00) regardless of BAP. At 5 mg L^{-1} GA_3, the number of roots increased by 37% at 3.5 mg L^{-1} BAP, by 149% at 4.5 mg L^{-1} BAP, and by 100% at 6.5 mg L^{-1} BAP compared with the same BAP level at 4 mg L^{-1} GA_3, whereas no increase occurred at 2.5 and 5.5 mg L^{-1} BAP. Averaged over the GA_3 levels, 4.5 mg L^{-1} BAP gave the highest number of roots (1.56), followed by 6.5 mg L^{-1} BAP (1.50), and averaged over the BAP levels, 5 mg L^{-1} GA_3 produced 57% more roots (1.47) than 4 mg L^{-1} GA_3 (0.93).

Table 5. Interaction effect of BAP and GA_3 concentrations on the number of roots of mung bean plantlets

BAP concentration	G1 (GA_3 4 mg L^{-1})	G2 (GA_3 5 mg L^{-1})	Mean BAP	Change G2 vs G1 (%)
B1 (2.5 mg L^{-1})	0.89 b	0.89 b	0.89	0
B2 (3.5 mg L^{-1})	0.89 b	1.22 b	1.06	+37
B3 (4.5 mg L^{-1})	0.89 b	2.22 a	1.56	+149
B4 (5.5 mg L^{-1})	1.00 b	1.00 b	1.00	0
B5 (6.5 mg L^{-1})	1.00 b	2.00 a	1.50	+100
Mean GA_3	0.93	1.47	1.20	+57

Note: Treatment means followed by the same letter are not significantly different according to DMRT at the 5% level. Marginal means were calculated from the treatment means.

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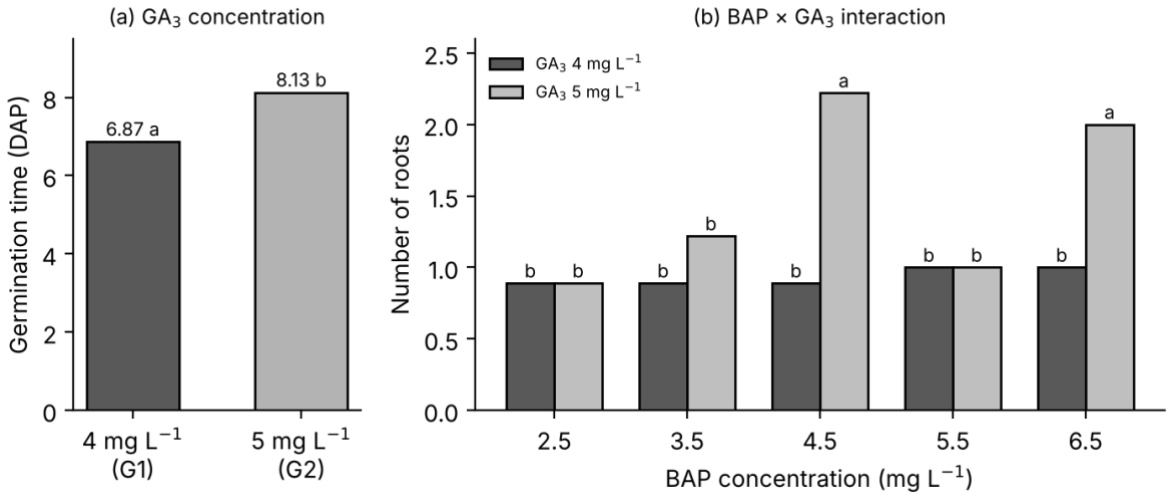

Figure 1. (a) Germination time of mung bean seeds at two GA_3 concentrations and (b) number of roots per plantlet for the ten BAP × GA_3 combinations; different letters indicate significant differences according to DMRT

Table 6 summarizes the treatments that gave the best response for each variable. The fastest germination was obtained with 4 mg L^{-1} GA_3, whereas the largest number of roots was obtained with 5 mg L^{-1} GA_3 combined with 4.5 mg L^{-1} BAP. No single combination was optimal across all variables, and for four of the six variables, no treatment was superior to the others. The choice of medium therefore depends on whether rapid germination or better rooting is the main objective of the culture stage.

Table 6. Summary of the best treatments for each observed variable

Variable	Best treatment	Value	Effect
Germination time	G1 (GA_3 4 mg L^{-1})	6.87 DAP	GA_3 **
Number of roots	B3G2 (BAP 4.5 + GA_3 5 mg L^{-1})	2.22 roots	B × G *
Plant height (32 DAP)	No difference	–	ns
Number of leaves (32 DAP)	No difference	–	ns
Stem diameter	No difference	–	ns
Plantlet dry weight	No difference	–	ns

Note: ** = highly significant ($p < 0.01$); * = significant ($p < 0.05$); ns = not significant. B5G2 (BAP 6.5 + GA_3 5 mg L^{-1}; 2.00 roots) did not differ significantly from B3G2.

Plant height, number of leaves, stem diameter, and plantlet dry weight at the end of the culture period did not differ significantly among BAP concentrations, GA_3 concentrations, or their combinations (Table 3). This indicates that, within the ranges tested, shoot growth and biomass accumulation were similar across all media, and that the treatments primarily affected germination speed and root formation.

DISCUSSION

This study showed that GA_3 and BAP affected different stages of mung bean in vitro growth. GA_3 concentration controlled germination rate, whereas root formation depended on the combination

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of BAP and GA_3. Shoot growth, expressed as plant height, number of leaves, and stem diameter, as well as plantlet dry weight, did not respond to the treatments within the range of concentrations tested. These results partly support our hypothesis: GA_3 affected germination, and the interaction affected roots, but not shoots.

The faster germination at 4 mg L^{-1} GA_3 than at 5 mg L^{-1} GA_3 indicates that the lower concentration was closer to the optimum for mung bean seeds. Gibberellins promote germination by weakening the tissues surrounding the embryo, counteracting abscisic acid, and inducing hydrolytic enzymes such as α-amylase that convert starch reserves into sugars for embryo growth (Tuan et al., 2018; Vishal and Kumar, 2018). The balance between GA and ABA, rather than the absolute GA level alone, determines when germination begins (Tuan et al., 2018). When the exogenous GA_3 concentration exceeds the seed's requirement, additional GA_3 does not further accelerate germination and may disturb hormonal balance. Mung bean seeds are generally not deeply dormant, so their endogenous gibberellin content may already be sufficient for germination, and a small external supply is enough to promote it. Similar concentration-dependent responses have been reported in other species, in which moderate GA_3 concentrations improved germination, whereas higher concentrations provided no additional benefit (Millones-Yamunaqué et al., 2021).

Another factor that may explain the slower germination at 5 mg L^{-1} GA_3 is the presence of BAP in all media. Cytokinins and gibberellins often act antagonistically: cytokinin can reduce GA responses, while GA can suppress cytokinin signaling (Velandia et al., 2024; Ma et al., 2024). In our experiment, all seeds were exposed to relatively high BAP concentrations (2.5–6.5 mg L^{-1}). The higher GA_3 concentration may have altered the overall hormonal balance, slightly delaying radicle emergence. Because no medium without BAP or GA_3 was included, the absolute effects of each regulator compared with a hormone-free control cannot be determined from this experiment. Including a control without growth regulators would allow separation of $GA_{3's}$ effect from that of the BAP background.

The significant interaction for the number of roots indicates that GA3's effect on rooting depended on BAP concentration. Root formation is controlled by a network of hormones, with auxin playing a central role, while cytokinin and gibberellin modulate meristem activity and cell elongation (Ma et al., 2024). Cytokinins usually promote shoot formation and inhibit root initiation when applied at high concentrations, because they shift the cytokinin-to-auxin ratio towards shoot development (Hnatuszko-Konka et al., 2021). Gibberellins, on the other hand, regulate the size of the root meristem and the elongation of root cells (Rizza et al., 2021; Shtin et al., 2022), and GA produced in the endodermis promotes the formation of lateral root organs in legumes (Velandia et al., 2024). The higher number of roots at 5 mg L^{-1} GA_3, combined with 4.5 or 6.5 mg L^{-1} BAP, suggests that the higher GA_3 concentration partly counteracted BAP's inhibitory effect on rooting, allowing more roots to develop.

The response, however, was not linear with BAP concentration. The increase in roots at 5 mg L^{-1} GA_3 was large at 4.5 and 6.5 mg L^{-1} BAP, small at 3.5 mg L^{-1} BAP, and absent at 2.5 and 5.5 mg L^{-1} BAP. This irregular pattern indicates that the root response was sensitive to small differences in hormone balance and possibly also to variation among individual seeds. The number of roots was low overall (0.89–2.22 per plantlet), which is typical of cytokinin-rich media without auxin. Rathore et al. (2023) found that BAP was the most effective regulator for multiple shoot regeneration in mung bean, but regenerated shoots normally require a separate rooting step. In another study on mung bean, roots were produced only in cultures fortified with the auxin IBA (Iqbal et al., 2022). These findings suggest that adding a low concentration of auxin or reducing the BAP concentration would be more effective for improving rooting than increasing GA_3 alone.

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The importance of the cytokinin–gibberellin balance for root development in mung bean is supported by studies in whole plants. Under waterlogging stress, GA_3 application mainly increased shoot growth, whereas cytokinin mainly improved root development in mung bean (Islam et al., 2021), and both hormones improved biomass and photosynthesis (Islam et al., 2022). In common bean, foliar GA_3 increased stem height and root dry mass, while seed treatment with GA_3 did not change the number of leaves or leaf area (Jaques et al., 2019). These results show that the effects of both hormones depend strongly on concentration, method of application, and developmental stage.

Plant height, number of leaves, stem diameter, and dry weight were not affected by the treatments. Several factors may explain this. First, the concentrations tested were relatively high and within a narrow range: BAP varied from 2.5 to 6.5 mg L^{-1}, and GA_3 only from 4 to 5 mg L^{-1}. All media therefore contained strong cytokinin and gibberellin signals, which may have saturated the growth responses. Second, during the first weeks of growth, seedlings depend largely on the reserves stored in the cotyledons, so differences in the medium have only a small effect on biomass within 32 days. Third, high cytokinin concentrations in vitro can reduce shoot quality and cause hyperhydricity, which may mask the positive effects of the regulators (Ersali, 2024). Studies on common bean also showed that in vitro responses depend strongly on the explant type and hormone concentration (Haliloğlu et al., 2023).

From a practical perspective, the results suggest a two-step approach for producing mung bean planting material in vitro. A medium containing 4 mg L^{-1} GA_3 can be used to achieve rapid, uniform germination, thereby shortening the time required to produce clean seedlings for further multiplication. For plantlets to be transferred to ex vitro conditions, the combination of 4.5 mg L^{-1} BAP and 5 mg L^{-1} GA_3 produced the highest number of roots. A well-developed root system is crucial for the survival of plantlets during acclimatization, because plantlets must take up water and nutrients as soon as they leave the culture vessel (Mohammed et al., 2023). Producing plantlets with more roots should therefore improve survival after transfer and the efficiency of the whole propagation system (Delgado-Paredes et al., 2021).

In the context of legume improvement, efficient in vitro protocols are also needed to support the multiplication of selected genotypes and the application of modern breeding tools (Dwivedi et al., 2023; Kanishka et al., 2023). Because mung bean is valued for its high nutritional quality (Hou et al., 2019; Mehta et al., 2021), the rapid production of uniform planting material from superior varieties can help increase both productivity and the supply of nutritious food.

The results also show that different stages of in vitro development may require different hormone balances. Germination was fastest at the lower GA_3 concentration, whereas root formation was best at the higher GA_3 concentration combined with an intermediate or high BAP concentration. In a single-step culture, the medium must therefore balance these partly conflicting requirements. A two-step system, in which seeds are first germinated on a medium optimized for germination and then transferred to a medium optimized for shoot and root development, may yield better overall results. Such stage-specific media are commonly used in micropropagation protocols for legumes, in which shoot induction with cytokinin is followed by a separate rooting stage (Rathore et al., 2023; Iqbal et al., 2022).

It should also be noted that the number of roots in this study was low in absolute terms, with even the best treatment producing just over two roots per plantlet. Plantlets with two or three roots may still survive acclimatization if the roots are healthy and well-developed, but a larger, more branched root system would increase the chance of survival. Root length, root branching, and root dry weight were not measured in this study, and these variables would provide a more complete

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picture of root quality. Measuring them in future experiments would allow a better evaluation of the practical value of the BAP and GA_3 combinations for producing planting material. In addition, the relationship between root number and survival after transfer to soil should be tested directly, because in vitro root traits do not always predict ex vitro performance (Mohammed et al., 2023).

The low and variable number of roots also highlights the importance of the experimental conditions. In vitro root formation is influenced by the sucrose and mineral content of the medium, the light conditions, and the gas exchange of the culture vessel, as well as by plant growth regulators. High humidity and ethylene accumulation in closed vessels can reduce root development and favor hyperhydricity, particularly in media with high cytokinin concentrations (Ersali, 2024). Improving vessel ventilation and reducing cytokinin concentration in the later stages of culture could therefore improve plantlet quality.

This study has several limitations. The concentration ranges of BAP and GA_3 were narrow, no hormone-free control was included, only one variety was tested, and the plantlets were observed for only 32 days without evaluating acclimatization. In addition, the number of roots was the only growth variable that responded to the treatments. Future studies should include a hormone-free control, wider and lower concentration ranges, testing the addition of auxin for rooting, evaluating root length and quality, and assessing the survival of plantlets after acclimatization.

CONCLUSION

GA_3 concentration significantly affected the in vitro germination of mung bean, with 4 mg L^{-1} GA_3 producing faster germination (6.87 DAP) than 5 mg L^{-1} GA_3 (8.13 DAP), while the interaction between BAP and GA_3 significantly affected the number of roots, with the highest values in the combinations of 5 mg L^{-1} GA_3 with 4.5 mg L^{-1} BAP (2.22 roots) and 6.5 mg L^{-1} BAP (2.00 roots). Plant height, number of leaves, stem diameter, and plantlet dry weight were not affected by the treatments. A medium containing 4 mg L^{-1} GA_3 is therefore recommended for rapid germination, and the combination of 4.5 mg L^{-1} BAP and 5 mg L^{-1} GA_3 for producing mung bean plantlets with better root formation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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