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# Exogenous application of brassinolide affects ripening, phenotypic traits, jelly seed and sensory properties of 'Dashehari' mango at ambient storage

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## ABSTRACT

*The present study aimed to elucidate the effects of brassinolide (BRs) on physical traits, jelly seed index and sensory properties of mango fruits. For this, BRs in different concentrations (20, 40, 60 and 80 ng/g) were applied to freshly harvested 'Dashehari' mango fruits through dip method. Control fruits were dipped in double distilled water. Treated and control fruits were stored at ambient conditions. The results suggested that BRs showed a concentration-specific behaviour over the fruits during storage. Lower doses accelerated the ripening process whereas higher doses delayed the ripening process. BRs (80 ng/g) treated fruits took a maximum (4.5 d) to ripen and significantly retained the peel colour, peel thickness (1.22 mm), pulp colour index (5.25), greenness index (2.5) and specific gravity (0.99). Likewise, fruits treated with BRs (80 ng/g) did not impair the tissue firmness and such fruits did not exhibit jelly seed symptoms. The fruits of same treatment were significantly assigned a higher sensory score compared to the control after 9 days of storage at ambient conditions. Hence, BRs (80 ng/g) was found to be suitable in delaying ripening and retaining the phenotypic characteristics without jelly seed symptoms.*

**Keywords:** Peel thickness; Quality; Colour; Aroma; Storage

## INTRODUCTION

Mango (*Mangifera indica* L.) is considered as choicest tropical fruit due to its high nutritional value, pleasant aroma and enormous health benefits (Meena and Asrey, 2018a). It is a popular fruit of Asian countries and placed almost at the top position in the tropical world. Certain commercial cultivars such as Amrapali, Dashehari, Alphonso, Langra, Chounsa etc. are popular in North India and neighbouring countries like Pakistan, Bangladesh and Nepal. Nevertheless, 'Dashehari' is quite the choicest cultivar due to its juicy and delicious taste. 'Dashehari' cultivar is most popular in North India and neighbouring countries like Bangladesh, Pakistan and Sri Lanka. Being climacteric, fruits are prone to rapid deterioration at room temperature. Elevated ethylene and respiration rate during the onset of ripening limits its shelf life (Singh *et al.,* 2013). Fruits are hardly fit for consumption for up to 5-7 days. Certain pre and postharvest factors are responsible for the regulation of ripening and food value (Meena and Asrey, 2018b). Application of edible coatings, postharvest fungicide treatments, hot water treatment, advanced storage technology and intelligent packaging are common practices being used to preserve fruit quality for the past couple of years. Despite that, implementation of 'Green Technology' and safer approaches are in the infant stage and need to be addressed. Efforts are being continued to develop sustainable, eco-friendly and safe approaches (Ntsoane *et al.,* 2019). Recently significant achievements have been made to regulate the ripening and metabolic process through the use of novel hormonal approaches. Moreover, the use of novel phytohormones such as brassinosteroids (BRs) provided a path to elucidate the mechanism and its crosstalk effects in the ripening process to extend the shelf life and quality. Brassinosteroids are a group of steroid plant hormones and are considered as 'sixth group of hormones' (Baghel *et al.,* 2019). Studies suggested that 24-Epibrassinolide and brassinolide are the most active form of Brassinosteroids. It has a pleiotropic role in the plant system from germination to senescence. Researchers have obtained exciting results of exogenous BRs application on the ripening behaviour of fruits. Zaharah et al. (2012) have found accelerated ethylene and advanced ripening in BRs-treated mango fruits whereas Pakkish et al. (2019) obtained contradictory

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results in grapes where BRs treatments delayed the ripening and enhanced the shelf life of grape fruits. Certain results suggested that the mechanism of BRs in the regulation of ripening in fruits is still unclear and needs to be validated. We have tried to standardise the optimum concentration of BRs in mango based on some phenotypic characteristics, jelly seed and sensory traits of the fruit.

## MATERIALS AND METHODS

Freshly harvested commercially mature mango fruits (SG~0.99, TSS~9 °Brix) were cleaned and de-sapped with distilled water. Fruits were divided into separate lots containing 30 fruits in each dipped in various doses of BRs viz. (20, 40, 60 and 80 ng/g) with a control (distilled water). Treated fruits were kept in cardboard fibre boxes in a single layer and stored at ambient conditions. The observations were taken on the initial day and at the end of the experiment i.e., the $9^{th}$ day. Days taken to ripen were calculated based on surface colour changes, firmness and pulp colour. The greenness index (GI) of the surface was calculated by the method previously followed by Gad *et al*. (2013). A score from 0 to 4 was generated based on the intensity of colour according to the following index: 0: 100% green; 1: 1-25% yellow; 2: 26-50% yellow; 3: 51-75% yellow and 4: 76-100% yellow. The specific gravity (SG) of fruits was determined by dipping fruits into a water Eureka can and the displacement of water was measured. The weight of the fruit was divided by the volume. Similarly, the score for the pulp colour index was generated from 1 to 6 based on the changes in pulp colour. The Peel thickness of fruits was measured by a vernier calliper and expressed in mm (Gupta *et al.,* 2023).

The jelly seed index of fruits was measured by observing visual symptoms. The disintegrated portion of halved fruits was measured and assigned a score as per the severity of incidence. According to the incidence area, fruits were divided into the slight (0-25%), moderate (25-50%), severe (50-75%), extreme (75-100%) and no symptoms categories. Sensory evaluation after the $9^{th}$ day of storage was done by using a Hedonic scale pattern. For this, a trained panel of 20 people tasted the fruits for colour, aroma, sweetness, sourness and overall acceptability and assigned a score from 1-9 which shows 1: extremely dislike and 9: extremely like (Ghasil *et al.,* 2023).

## STATISTICAL ANALYSIS

All the data of stored mango samples were collected and subjected to analysis of variance by using CRD design with a software WSAP version 2.0 for windows. The average value of each parameter has four replicates (n=4). The mean comparison was carried out by Tukey's HSD test.

## RESULTS AND DISCUSSION

### *Days taken to ripen*

Days taken to ripen were significantly delayed by the exogenous BRs treatments (Fig 1). The fruits treated with BRs (80 ng/g) took a maximum of 4.5 days to ripen. However, lower doses accelerated the ripening process compared to higher doses and control. Ripening is a continuous process in climacteric fruits and is triggered by ethylene. BRs at higher concentrations delayed the ethylene biosynthesis process and slowed down the metabolic activities in mango.

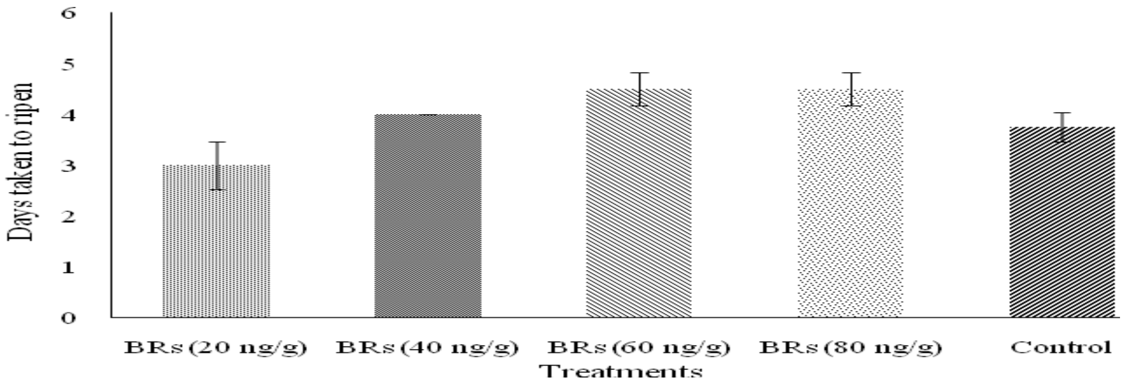


Fig 1: Effects of exogenous brassinolide on days taken to ripen of 'Dashehari' mango fruits. The vertical bar represents mean± standard error of four replicates (n=4)

Earlier Pakkish *et al*. (2019) also reported that BRs application delayed the ripening process in grape fruits.

***Physical parameters***

Changes in the greenness index (GI) of the fruit surface and specific gravity (SG) are shown in Table 1. During the initial day, a non-significant value of GI and SG was recorded. During the storage period, significant changes were observed in both the GI and SG. During ripening greenness was reported to be reduced and at higher concentrations (BRs 80 ng/g) fruits got a minimum GI score (2.5) and SG (0.99).

Table 1: Effect of exogenous brassinolide on greenness index (GI) and specific gravity (SG) of 'Dashehari' mango fruits during ambient storage

| Treatments | GI | | SG | |
|---|---|---|---|---|
| | Storage (days) | | | |
| | 0 | 9 | 0 | 9 |
| BRs (20 ng/g) | 1.0* | 3.75[a] | 0.97* | 1.01[a] |
| BRs (40 ng/g) | 1.0* | 3.5[ab] | 0.98* | 1.00[ab] |
| BRs (60 ng/g) | 1.0* | 3.25[abc] | 0.98* | 1.01[abc] |
| BRs (80 ng/g) | 1.0* | 2.5[c] | 0.97* | 0.99[c] |
| Control | 1.0* | 2.75[bc] | 0.98* | 1.00[bc] |

*Data represents mean of four replicates (n=4). The similar letters are statistically non-significant at 5% (p≤0.05) level of significance. Star indicates non-significant value in a single column. Abbr. GI: Greenness index; SG: Specific gravity*

Lower concentration did not preserve the green colour of the fruit surface compared to the control. The degradation of green pigment chlorophyll into carotenoids is a common process in mango. However, in the 'Dashehari' cultivar, the least colour changes take place during the ripening process and a slight colour variation can be seen over the fruit surface (Gupta *et al.,* 2023). The minimum GI score in fruits could be attributed to the delayed degradation process of pigments. In addition to that, lower ethylene evolution in treated fruits could have slowed down the breaking stage in mango. However, a contradictory result was also reported by Zhu *et al.* (2015) in tomatoes where BRs treatments significantly decreased chlorophyll content and enhanced lycopene content. Generally, SG increases in fruits with the advancement of the ripening process (Meena and Asrey, 2018a). Our results suggested that BRs (80 ng/g) delayed the ripening process thus resulting in lower respiration and ethylene evolution. The pore space among the cells becomes dense during the ripening process. As a result of catabolic and anabolic changes cell arrangement become dense and filled with soluble materials. That could be a reason for SG enhancement. Earlier Meena and Asrey (2018a &b) reported enhanced specific gravity in Amrapali mango.

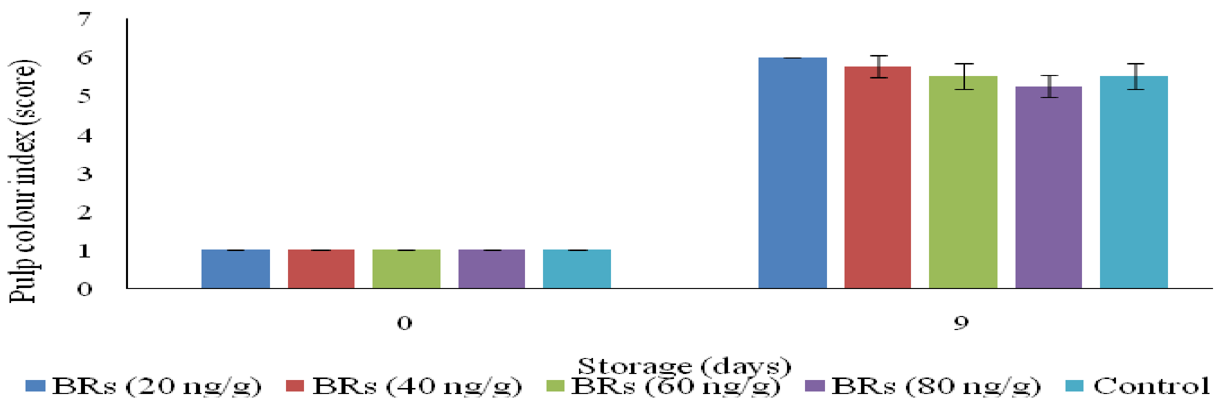


Fig. 2: Effects of exogenous brassinolide on pulp colour of 'Dashehari' mango fruits during storage at ambient conditions. The vertical bar represents mean± standard error of four replicates (n=4)

Pulp colour was significantly (P≤0.05) influenced by the application of BRs (Figure 2). As in the case of other parameters, pulp colour showed a similar trend. At a higher dose, changes in pulp colour were significantly delayed but lower concentration did not preserve colour loss. The results have shown that BRs (80 ng/g) effectively preserved the colour of pulp and assigned a better score (5.25) compared to other treatments. It is reported that BRs treatment inhibits chlorophyll degrading enzymes, and maintains chloroplast structure and ethylene synthesis enzymes which retain firmness and colour (Cai *et al.,* 2019). Nevertheless, accelerated ethylene biosynthesis and carotenoids are responsible for the yellow to the pinkish colour of mango pulp. During the ripening process colour starts changing from dull to yellowish. A high dose of BRs showed positive impacts on pulp firmness and colour during 9 d of storage.

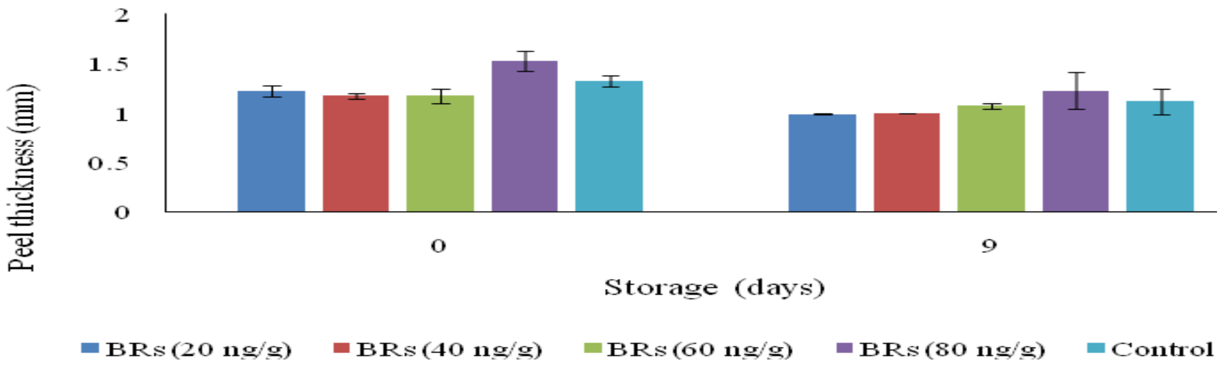


Fig 3: Effects of brassinolide on peel thickness of 'Dashehari' mango fruits during storage at ambient conditions. The vertical bar represents mean± standard error of four replicates (n=4)

Variation in peel thickness of mango fruits as affected by BRs treatments is shown in Figure 3. Peel thickness has shown a decreasing trend during storage time in all the treatments. Concerning treatments, BRs (80 ng/g) significantly showed better peel thickness (1.22 mm) compared to other treatments and control. Fruit peel is mainly composed of cellulose, hemicellulose and pectic substances. Calcium pectate is found in the insoluble form converts into soluble pectin as a result of pectin methyl esterase, cellulase and polygalacturonate enzymes actions. BRs (80 ng/g) might have induced the activities of defence-related enzymes and suppressed the activity of pectic enzymes thus reducing the changes in the peel thickness of mango fruits. In contrast, EBRs treatments enhanced the softening-related enzymes in persimmon and brassinazole delayed the activities of softening enzymes (He *et al.,* 2018).

### *Jelly seed*

The Jelly seed index in 'Dashehari' mango fruits affected by various concentrations of BRs is shown in Table 2.

Table 2: Occurrence of jelly seed symptoms in brassinolide treated 'Dashehari' mango fruits after 9$^{th}$ day of storage

| Treatments | Severity of symptoms |
|---|---|
| BRs (20 ng/g) | Moderate |
| BRs (40 ng/g) | Moderate |
| BRs (60 ng/g) | Slight |
| BRs (80 ng/g) | No symptoms |
| Control | Slight |

Based on the affected area, a classification was made for the jelly seed index. It was interesting to note that BRs (80 ng/g) did not show any symptoms related to tissue disintegration. On the other hand, BRs doses such as 20 and 40 ng/g did not prevent jelly seed formation and fruits exhibited moderate symptoms of jelly seed formation (25-50%). 'Dashehari' mango is very prone to jelly seed formation and the incidence of jelly seed in 'Dashehari' ranges from 21.3 to 36% in artificially ripened and tree-ripened fruits respectively (Srivastav *et al.,* 2015). The higher dose of BRs helps in retaining the integrity of pulp and likewise delayed the softening enzymatic activity. Thereby, the pulp tissue retained firmer and did not show the symptoms of jelly seed. Our results also suggested that lower concentration of BRs accelerated ripening and induced the pectolytic enzymatic activities (data not included) thus more tissue disintegration appeared in such fruits.

Table 3: Effect of exogenous brassinolide on sensorial properties of 'Dashehari' mango at the end of ambient storage

| Treatments | Sensory score | | | | |
|---|---|---|---|---|---|
| | Colour | Aroma | Sweetness | Sourness | Overall acceptability |
| BRs (20 ng/g) | $4.4^{c}$ | $2.8^{d}$ | $3.2^{c}$ | $3.4^{c}$ | $3.6^{d}$ |
| BRs (40 ng/g) | $4.8^{c}$ | $3.4^{d}$ | $3.8^{bc}$ | $4.0^{bc}$ | $4^{cd}$ |
| BRs (60 ng/g) | $4.4^{c}$ | $4.4^{c}$ | $4.6^{b}$ | $4.4^{b}$ | $4.6^{c}$ |
| BRs (80 ng/g) | $7.2^{a}$ | $7.4^{a}$ | $7^{a}$ | $5.8^{a}$ | $7^{a}$ |
| Control | $6^{b}$ | $6.0^{b}$ | $6.6^{a}$ | $6.0^{a}$ | $6^{b}$ |

*Data represents mean of sensory score given by a trained panel of experts according to Hedonic scale (range 1-9). The similar letters are statistically non-significant at 5% (p≤0.05) level of significance*

### *Sensory evaluation*

As shown in Table 3, only BRs (80 ng/g) treated fruits and control fruit showed substantial sensory scores which were under the acceptable limit. Among the treatments, BRs (80 ng/g) fruits exhibited better sensory scores for colour (7.2), aroma (7.4), sweetness (7.0), sourness (5.8) and overall acceptability (7.0) followed by control on the 9$^{th}$ day of storage at ambient condition. On other hand, the least score was assigned to the fruits treated with BRs (20 ng/g) for all the above-mentioned sensory parameters. Sensory parameters are the consumer parameters that determine the acceptability of a particular commodity. The treatments-maintained sugars, TSS, organic acids and pigments that might be affected the sensory attributes during storage and helped in securing higher scores by the panel. Several other hormonal treatments such as methyl jasmonate (MeJA) and salicylic acid (SA) retained higher sensory scores in Kinnow (Dhami *et al.,* 2022).

## CONCLUSION

The present investigation indicated that BRs significantly affected the various phenotypic characters of 'Dashehari' mango fruits however, the results are specific concentration dependent. Present results revealed that the application of BRs (80 ng/g) exhibited better physical parameters and sensory scores without developing any jelly seed symptoms. Further, based on the findings it can be speculated some possible future avenues to draw a conclusion about its mechanism and biological activities and a particular dose can be standardized for a climacteric crop like mango.

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