Exogenous gibberellic acid does not induce early flowering in mungbeans
[Vigna radiata (L.) Wilczek.]

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ABSTRACT
Gibberellic acid (GA) is an important phyto-hormone mediating plant growth. In present study, we evaluated the impact of GA treatment on morphological, phenological and molecular aspects of five mungbean genotypes. GA treatment caused a significant increase in plant height and branch angle in most of the genotypes. However, there was no impact of GA treatment on days to first anthesis, days to 50% flowering and pod length. Genotypes SML–859 and EC-48 revealed no change in their plant height and branch angle respectively upon GA treatment, suggestive of their probable GA insensitivity. Expression of flowering associated gene- VrSOC1 remained unaffected by GA treatment, validating thereby that exogenously supplied GA does not induce early flowering in mungbeans.

Key words: Branch angle, Gene expression, Genetic variability, Plant height, Pod length.

INTRODUCTION
Mungbean [Vigna radiata (L.) R. Wilczek var. radiata] is a grain legume belonging to genus Ceratotropis of the Fabaceae family. It is a self-pollinated diploid species with a chromosome number of 2n= 22 and a genome size of 543 Mb. The crop is vital to the Asian farmers with small land- holdings with an annual production of 3.5- 4.0 million tonnes. Mungbean seed is rich in carbohydrates, protein (27%), folate and iron (Noble et al., 2017). It is an important source of protein in South and Southeast Asia (Somta et al., 2016) and is consumed as a soup of split seeds and spices called Dal or Dhal. India is largest producer and consumer of mungbean, covering an area of 4.3 million haactare with the production of 2.07 million tonnes and productivity of 481 Kg/ha (Anonymous 2017). The crop fits well in multi-cropping systems, because of its rapid growth and early maturity. Since the crop is widely grown in marginal and abiotically stressed agro-ecosystems (Raina et al., 2016), it experiences considerable yield losses. Abiotic stress factors like flooding, heat and drought inflict heavy damage to crop productivity world-wide. However, plants being sessile have developed enormous diversity in their intrinsic factors which confers survival under wide range of environments. Hormones are important chemical messengers which modulate plant growth in response to environmental cues. Although, response to a particular stress condition is governed by a specific hormone, the overall response is in part regulated by the extensive crosstalk among various hormones. Moreover, it is not only the variability in endogenous levels of a hormone but also proportions which contribute to stress adaptation. Genetic diversity in sensitivity to phyto-hormones also plays an important role in differential stress responses.

Among the several plant hormones, gibberellic acid is an important one mediating growth in plants (Hernandez 1997 and Awan et al., 1999). Gibberellins induce early flowering in a variety of plants especially long day plants (Lang 1956). However, reports of Gibberellins inducing early flowering in short day plants are few (Kumar et al., 1977). Gibberellic acid induced faster bud development in Ajania pacifica (Zalewska and Antkowiak 2013). Arabidopsis plants exogenously treated with GA flower earlier, particularly under short days (Blázquez et al., 2002). GA biosynthesis is also known to play an important role in mediating ethylene induced repression of flowering genes LEAFY and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (Grauwe et al., 2008). GA3 treatment also induced early inflorescence emergence in Miltoniopsis orchid hybrids (Matsumoto 2006). Moreover, in plant species like Arabidopsis and Tomato, gibberellic acid deficiency leads to male sterility (Goto and Pharis 1999). With this background, we explored the effect of GA treatment on flowering in mungbeans, a short day plant (Sardana et al., 2010). GA treatment induced significant increase in plant height and branch angle in most of the genotypes. However,
Table 1: Details of primers used in current study

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Primer Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>VrActin</td>
<td>VrACTrtF</td>
<td>CCCGAAGTTCTGTTCCAGCCAAT</td>
</tr>
<tr>
<td></td>
<td>VrACTrtR</td>
<td>GTA TTTCCTCTCTGGTGGTGCG</td>
</tr>
<tr>
<td>VrSOC1</td>
<td>VrSOCrtF</td>
<td>CATAGAGAACGCCACAACAGCA</td>
</tr>
<tr>
<td></td>
<td>VrSOCrtR</td>
<td>GAGCAACCTCAGCATCAAA</td>
</tr>
</tbody>
</table>

**Materials and Methods**

**Growing conditions:** The experiment was conducted in summer of 2015 at the premises of ICAR-National Institute of Abiotic Stress Management, Baramati (Maharashtra). Mungbean genotypes used in the study were obtained from ICAR-IIPR and PAU, Ludhiana. The seeds were multiplied and plants raised from them were grown under natural environmental conditions in pots (28 cm X 22.5 cm) filled with a mixture of sand, silt and FYM in equal proportion. The required amount of N, P & K fertilizers were uniformly mixed with the soil before filling pots. The pots were arranged in a completely randomized design with 3 replications and watered regularly. The moisture content of the control pots was maintained around 70-75% of field capacity.

**Gibberellic acid treatment:** 500 ppm Gibberellic acid (G7645-5G, Sigma) was sprayed uniformly over the plants at 20 days after sowing (DAS).

**Morphological data collection:** Both the control and treated plants were evaluated for morphological features like plant height, branch angle, days to anthesis and pod length. The plant height from the soil surface to the tip of plant was measured in 10 randomly selected plants/replication using a 1m steel ruler. Pod length of 20 mature pods/replication was measured using a 30 cm ruler. Days to flowering were calculated as total number of days taken from sowing of the seed to first flower opening. Days to 50% flowering was calculated as number of days taken from sowing of seed to flowering in 50% of the plants. Branch angle (θ) was measured as the angle between main stem and the side branch (Fig 1). The angle value was obtained using a protractor keeping its base aligned with the main shoot.

**Gene expression analysis:** Leaf samples (30DAS) from 2nd fully expanded of at least 3 plants of a replication were mixed and used for RNA extraction. Total RNA was extracted from the leaves using RNeasy Mini kit (Qiagen, Valencia, CA). The quality of RNA was determined using a Shimadzu Spectrophotometer and first strand cDNA synthesized from 2 μg of total RNA using Revert-Aid H-minus cDNA synthesis kit (Thermoscientific). Expression studies were conducted for transcript accumulation of *Suppressor of overexpression of constans 1* (*SOC1*) gene using gene specific primers. RT-PCR reaction was performed in 40 cycles program (95 °C for 10 s, 58 °C for 30 s) on CFX 1000 instrument (Biorad), using IQ SYBR GREEN master mix (Biorad). The 2^ΔΔCT method (Livak and Schmittgen 2001) was used to normalize and calibrate transcript values relative to the endogenous mungbean Actin gene. Three biological replicates were used for the expression analysis. The primer sets used for amplifying different target genes are shown in (Table 1).

**Statistical analysis:** The data were subjected to analysis of variance according to the model for completely randomized design using an SPSS program (SPSS Inc, Chicago, IL). Student’s t-test was used to determine the significance of the differences between mean values of control and treated plants.

**Results and Discussion**

Gibberellic acid treatment induced morphological changes in mungbeans: GA induced significant morphological changes in mungbean plants (Fig. 2). A significant (p<0.5) increase in plant height among most of the genotypes was observed (Fig 3a). Genotype Vaibhav recorded maximum increase in plant height (~ 2-fold) upon treatment while other genotypes recorded a modest increase in their height upon GA treatment. Interestingly, there was no significant change in plant height of genotype SML-859 when treated with GA. Hormones are the chemical messengers with specific role in plant growth and
Among the plant hormones, GA is the one known to modulate the various aspects of plant development like germination, plant height and flowering. In fact, GA was discovered owing to the increased height of the maize plants infected with *Gibberella fujikuroi*. In mungbeans, we observed increased plant height in plants treated with GA. However, genotypic variability was observed for response to hormone treatment with maximum response being recorded in Vaibhav while no significant change in plant height of SML-859 plants was observed. Non-responsiveness of SML-859 to GA treatment needs to be investigated further for presence of a possible mutation in GA signaling which has implications for crop improvement in this legume crop.

GA treatment also induced a significant (p < 0.5) change in the branch angle among mungbean genotypes (Fig. 3b). Genotype ML-613 recorded a maximum increase of 15.83° followed by VC-3960-BB, Vaibhav and SML-859. However, no change in branch angle was recorded in EC-48 upon GA treatment. Increased branch angle is expected to alter leaf orientation in way facilitating enhanced interception of the Photo-synthetically Active Radiation (PAR), although there are equally higher chances for increased shading of the lower leaves. Interestingly, in maize, smaller but flatter leaves around the ear are expected to contribute to increased tolerance to high density planting (Huang et al. 2017). Interestingly, auxins are known to specify the gravitropic setpoint angle (GSA) in plants (Roychoudhry et al. 2013) and GAs modulating the branch angle has not been reported.

We also evaluated the effect of GA treatment on pod length in all the five genotypes. However, there was no change in pod length in any of the genotypes upon GA treatment (Fig. 3c). Although exogenous application of GA3 is reported to increase the pod length in cowpea plants (Emongor 2007), it seems to have no impact on the pod length in mungbeans.

**Gibberellic acid induced no significant change in phenology and related gene expression:** Since GA is known to regulate flower initiation and its development, we investigated the effect of GA treatment upon flowering in mungbeans. No significant change in days to first anthesis was observed upon GA treatment among the genotypes used in study (Fig. 4a). Even days to 50% flowering remained unaffected by GA treatment (Fig. 4b). These observations
revealed that GA treatment does not influence the flowering in mungbeans.

Flowering is an important developmental phenomenon regulated by several cascades, each being controlled by several genes. SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1) is an important marker gene whose expression coincides with transition from vegetative meristem to inflorescence meristem, enabling commitment to flowering in Arabidopsis, a facultative LD plant (Mutasa-Gottgens and Hedden 2009). Therefore, we investigated the expression of Suppressor of overexpression of constans 1 (VrSOC1) gene in two mungbean genotypes ML-613 and VC-3960-BB exposed to GA treatment. Gene specific primers were designed using the gene sequence of VrSOC1 (KT025629) and transcript accumulation studied by quantitative RT-PCR using actin gene as reference. As seen from the picture (Fig. 5a & b), no significant change in the expression of SOC1 in two genotypes was observed upon GA treatment. Gibberellins are crucial for transition to reproductive growth (Hisamatsua et al. 2000). GA plays a role in regulating flowering under LD conditions (Griffiths et al., 2006; Willige et al., 2007) and is also suggested to have a role in floral transition under SD conditions (Li et al., 2017). The fact that no change in VrSOC1 expression was observed upon GA treatment, validated the unresponsiveness of flowering promotion to exogenous GA treatment in mungbeans.

CONCLUSION

These studies demonstrate the impact of exogenously supplied GA on morphology and phenological behavior in mungbeans. Interestingly, some genotypes like SML-859 and EC-48 with no significant change in plant height and branch angle respectively need to be further studied for potential mutations in GA signaling. Flowering was not influenced by GA treatment which was further supported by gene expression studies. The observation that GA treatment induced significant increase in branch angle has implications for the potential architectural manipulation of the crop involving GA signaling.

Author contribution statement: Susheel Kumar Raina (SKR) designed research, conducted most of the physiological and molecular experiments and data analysis. Punam Singh Yadav (PSY) and Ajay Kumar Singh (AKS) grew plant materials, helped in physiological and molecular biology experiments. Nikhil Raskar (NR) helped in gene expression and statistical analysis. Jagadish Rane (JR) advised in experimental design, helped in phenotyping and writing the manuscript. Paramjit Singh Minhas (PSM) coordinated the project and edited the manuscript. All authors read and approved the manuscript.

Conflict of interest: The authors declare no conflict of interest.

REFERENCES


