ISSN 1817-3721, E-ISSN 1818-8745

Plant Tissue Cult. & Biotech. **29**(2): 245-255, 2019 (December)

PTC&B

In Planta Genetic Transformation of Mungbean (*Vigna radiata* (L.) Wilczek) with Marker Gene

Mohammad Mahmood Hasan, Sujay Kumar Bhajan, M. Imdadul Hoque, R. H. Sarker and Mohammad Nurul Islam*

Plant Breeding and Biotechnology Laboratory, Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

Key words: Mungbean, In planta transformation, *Gus* gene, pBI121, Recalcitrant crop

Abstract

In genetic improvement of mungbean much success has not been achieved due to its recalcitrant nature towards *in vitro* regeneration. An attempt was made to develop an *Agrobacterium*-mediated in planta genetic transformation protocol for a locally grown mungbean variety BARI Mung-3 using a screenable marker gene. Two minutes of vacuum infiltration followed by 60 minutes of incubation period in *Agrobacterium* suspension of Winans' AB medium containing wounded tobacco leaf extract was found most suitable towards genetic transformation in pricked de-coated half seed explants. An optical density (OD_{600}) of 0.7 was found most effective for transient *gus* gene expression. Chimeric GUS expression was observed in the root and leaf tissues from the successfully transformed plantlets obtained through in planta transformation. This methodology of genetic transformation was found more suitable, easier and less time consuming than tissue culture based genetic transformation, which may be used for the genetic improvement of mungbean.

Introduction

Mungbean (*Vigna radiata* (L.) Wilczek) also known as green gram is a pulse crop mainly cultivated in south and south-east Asia, but the cultivation has also been extended to parts of USA, Canada, Australia and Ethiopia (Schafleitner et al. 2015). It is one of the major sources of vegetable protein (25%). It is also rich in carbohydrate, fat and fibre which made it an excellent supplement of cereal diets. Moreover, the early ripening characteristic, high nutritional value and easy digestibility have made its cultivation more popular (Yadav et al. 2012). In Bangladesh mungbean is the third most cultivated

*Author for correspondence: <m.nurul@du.ac.bd>.

DOI: https://doi.org/10.3329/ptcb.v29i2.44513

pulse crop with highest market value. Though it has high demand the production rate is far below. As a result, Bangladesh has to import a large amount of mungbean from its neighbouring countries (Bhajan et al. 2019).

The production of mungbean is further hindered by various abiotic (drought, temperature, water logging and salt) and biotic stress factors. Among biotic factors Mungbean Yellow Mosaic Virus (MYMV) is the most devastating one since it may result more than 50% yield loss (Vir et al. 2016, Islam and Islam 2010). Conventional breeding for genetic improvement of mungbean reported being a hurdle due to the lack of genetic variability as it is a self-pollinated crop and absence of resistant genes in the germplasm. Moreover, failure of pollen tube to penetrate stigma, failure in pod formation, embryo abortion lead to unsuccessful intervarietal hybridization (Vir et al. 2016, Yadav et al. 2010). Biotechnological approaches could help breeders to overcome such issues for the genetic improvement of mungbean since it allows integrating gene/s from distantly related organisms. However, a reproducible and efficient *in vitro* regeneration protocol is a pre-requisite of plant genetic transformation (Mohanty and Sagare 2015). Though successful *in vitro* regeneration of mungbean plantlets were reported from various explants, for instance, cotyledonary nodes (Mohanty and Sagare 2015, Vats et al. 2014, Himabindu et al. 2014), cotyledon, hypocotyl, root and shoot tip (Khatun et al. 2008), double cotyledonary node (Yadav et al. 2010), decapitated mature embryo (Hoque et al. 2007), cotyledon attached decapitated embryo (Bhajan et al. 2019) it is still considered as a recalcitrant plant towards tissue culture particularly in case of developing healthy and viable root system. Development of *Agrobacterium*-mediated genetic transformation protocol has also achieved very little success in mungbean as it is not amenable towards genetic modification (Vir et al. 2016, Dewir et al. 2016). Till date, very little success has been achieved towards the genetic modification of mungbean and most of them are confined with reporter gene (e.g. *gus*) (Bhajan et al. 2019, Yadav et al. 2012, Islam and Islam 2010, Tazeen and Mirza 2004, Jaiwal et al. 2001). There are some reports of successful development of transgenic mungbean with alien gene (Baloda and Madanpotra 2017, Kumar et al. 2017, Sahoo et al. 2016, Vijayan and Kirti 2012, Saini et al. 2007) but the success rate is reported to be between 1 and 2%.

Considering such recalcitrant nature of the mungbean plants towards *in vitro* regeneration, a tissue culture-independent genetic transformation method called in planta genetic transformation has been conceptualized. Such a method is quicker, simpler, cheaper, more efficient and somaclonal variation free (Rao et al. 2008, Niazian et al. 2017). Hence, attempts have been made to develop an *Agrobacterium*-mediated in planta genetic transformation protocol in a locally grown Bangladeshi mungbean variety, namely BARI Mung-3 using a screenable marker gene called "*gus*"gene.

Materials and Methods

Seeds of BARI Mung-3 (Pragati) developed and released by Bangladesh Agricultural Research Institute (BARI) on 1996 was used for in planta transformation. The seeds were

first washed with tap water and then surface sterilized with 70% alcohol (30 sec) and washed again with autoclaved distilled water immediately. Seeds were dipped in 0.1% $HgCl_2$ solution (8 - 10 min.) followed by 3 - 4 times wash in distilled water. Then the seeds were soaked overnight in autoclaved distilled water for germination in a dark chamber. The germinated seeds were then de-coated, and one of the cotyledons was removed carefully so that, the embryo remain attached with one cotyledon (half seed explants). For making injury into the explants, 0.5 ml insulin disposable syringe was used. Two to three pricks were made at the embryonal axis of the half seed explants to facilitate the transformation.

Agrobacterium tumefaciens strain LBA4404 (plasmid) was used harbouring in plant transformation vector of pBI121. The T-DNA portion between the right border (RB) and left border (LB) of the plasmid contains a *gus* gene flanked by CaMV 35S promoter and NOS terminator. The plasmid also has a kanamycin resistant gene (*nptII*) in the T-DNA segment which is driven by NOS promoter and terminated by NOS terminator (Fig. 1).

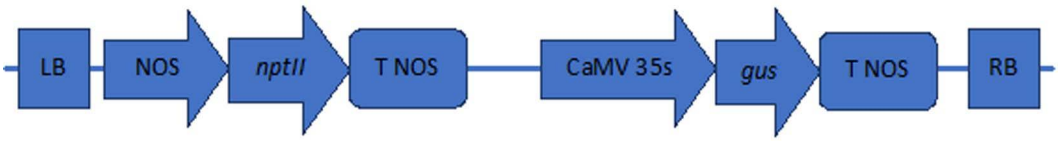

Fig. 1. Diagrammatic representation of the T-DNA region of the pBI121 plasmid.

The *A. tumefaciens* was grown overnight in 10 ml YMB medium supplemented with 50 mg/l kanamycin, 25 mg/l streptomycin and 25 mg/l rifampicin at 28°C in a rotary shaker at 250 rpm. On next day 1% culture was inoculated in 40 ml YMB medium and grown overnight in the above-mentioned conditions for antibiotic selections. This bacterial suspension was used directly to infect the explants at various optical densities (OD_{600}) to find out the optimum OD_{600} towards best transformation efficiency. The overnight cultured *Agrobacterium* in YMB medium was pellet downed by centrifugation at 5000 rpm for 10 min when the OD_{600} was 0.7. The pellet was re-suspended in Winans' AB medium (Winans et al. 1988) containing required antibiotics as mentioned earlier, followed by culture in an orbital shaker for around 18 hrs. For the induction of *vir* gene activity, fresh tobacco leaf extract (8 g of leaf was crushed in 8 ml of autoclaved distilled water) was added to the medium 5 hrs before the infection.

The de-coated half seed explants were injured at the point with a sterile 0.5 ml insulin disposable syringe and incubated in bacterial suspension in AB medium for different incubation periods (15, 30, 45, 60 and 120 min). Before incubation, the bacterial suspension containing the half seed explants were subjected to vacuum infiltration for different periods of time. After incubation, the explants were washed three times with autoclaved distilled water followed by 10 min of antibiotic wash (300 mg/l carbenicillin solution). After that explants were soaked dried on filter paper and then inoculated to half strength of MS, devoid of any kind of hormone and let them grow in tissue culture

growth room at 25°C and 16 hrs of light and 8 hrs of dark. After the formation of the healthy root system, the plantlets were washed with autoclaved distilled water to remove the adhering medium and then transferred to plastic pots containing autoclaved soil and hardened in tissue culture growth room conditions as mentioned earlier. The plants were transferred to larger earthen pots containing autoclaved soil.

The activity of *gus* gene expression was evaluated through GUS histochemical assay. *Agrobacterium* infected explants and different tissues (leaf, stem and root) were treated with X-gluc at 37°C for 72 hrs. Afterwards, the tissues were kept in 100% ethanol to bleach the chlorophyll contents. Some tissues were observed directly with naked eye and stereomicroscope (Nikon SMZ1000). Some tissues were hydrolyzed with 1N NaOH solution in a glass vessel at 65°C for 15 - 20 min until the tissues become soft enough to be macerated. The tissues were placed on the microscopic glass slide (with 50% v/v glycerol) and pressed with a cover slip to macerate the cells. The cells were observed under Nikon ECLIPSE 50i compound light microscope (Nikon, Japan).

Results and Discussion

The plant material (BARI Mung-3) used in this study was chosen because it has high yielding capacity (1000 to 1100 kg/ha), less cooking time (14 to 17 min) and 19 - 21% protein content but it has been reported to be susceptible to disease incidences like *Cercospora* leaf spot and MYMV (Faruq and Islam 2010). The de-coated half seeds are the best option for in planta transformation of pulse crops (Kapildev et al. 2016).

The efficiency of the *gus* gene transformation into plant tissue was evaluated through GUS histochemical assay (Jefferson et al. 1987) and the samples were chosen randomly. The successfully transformed cells developed blue colour within the cells of the half seed explants (Fig. 2), leaves, and root cells (Figs 3, 4). However, in most of the cases the expression pattern of GUS was found chimeric in nature. On the other hand, non-transformed tissues failed to develop blue colour in GUS histochemical assay. Such chimeric expression of gene is considered as a disadvantage in the in planta transformation method (Niazian et al. 2017). In order to eliminate non-transformed and chimeric transformed plants in case of in planta transformation BASTA® selection is proven to be most effective (Kapildev et al. 2016, Mayavan et al. 2015, Mayavan et al. 2013). In the present study such selection method was not used due to the unavailability of genetic construct with BASTA® resistant gene. Another study suggested that though T_0 generation showed chimeric expression of *gus* gene in T_1 generation the expression pattern becomes stable (Yellisetty et al. 2015).

To find out the impact of optical density, the explants were subjected to *Agrobacterium* culture of different optical densities at 600 nm (OD_{600}) for 30 min followed by GUS histochemical assay and OD_{600} at 0.7 was found to be most effective (Table 1). The transformation efficiency was found to be increased with the OD_{600} but up to a certain point. Optical density over 0.7 was found to decrease the transformation efficiency.

Fig. 2. In planta genetic transformation in BARI Mung-3. (a) Half seed explants on half strength MS, (b) well rooted *in vitro* germinated plants on half strength MS, (c) plants transferred to plastic pots, (d) hardened plant on earthen pot, (e) *In vivo* pod formation of the transformed plant and (f) successfully transformed half seed explants showing positive response along with control explants showing negative response in GUS histochemical assay.

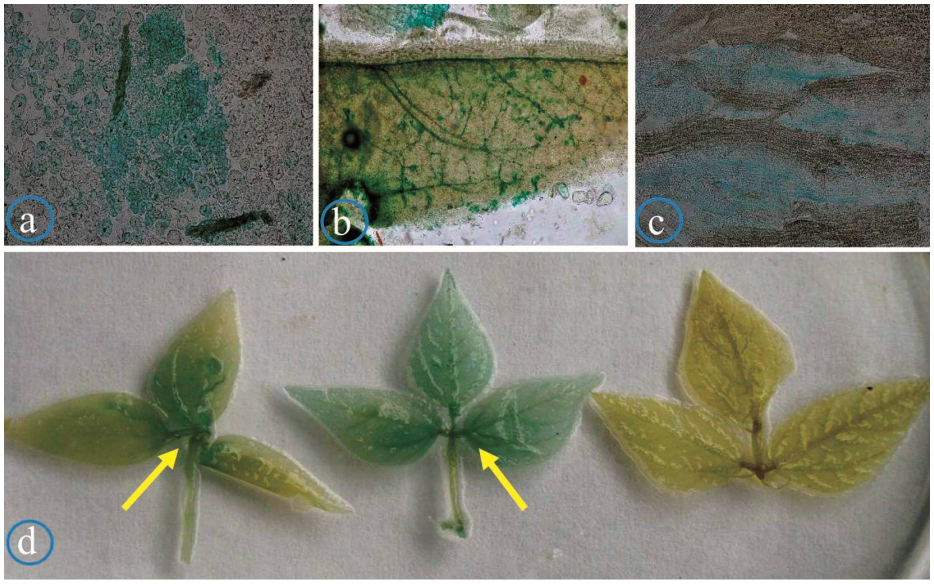

Fig. 3. GUS histochemical assay of the tissues of transformed mungbean plantlet. (a) Macerated cells of cotyledon, (b) macerated cells of plumule, (c) macerated cells of radicle tissues of half seed explants showing GUS positive response, (d) leaf of the transformed plants showing histochemical localization of GUS expression (blue colour) along with control showing no GUS expression.

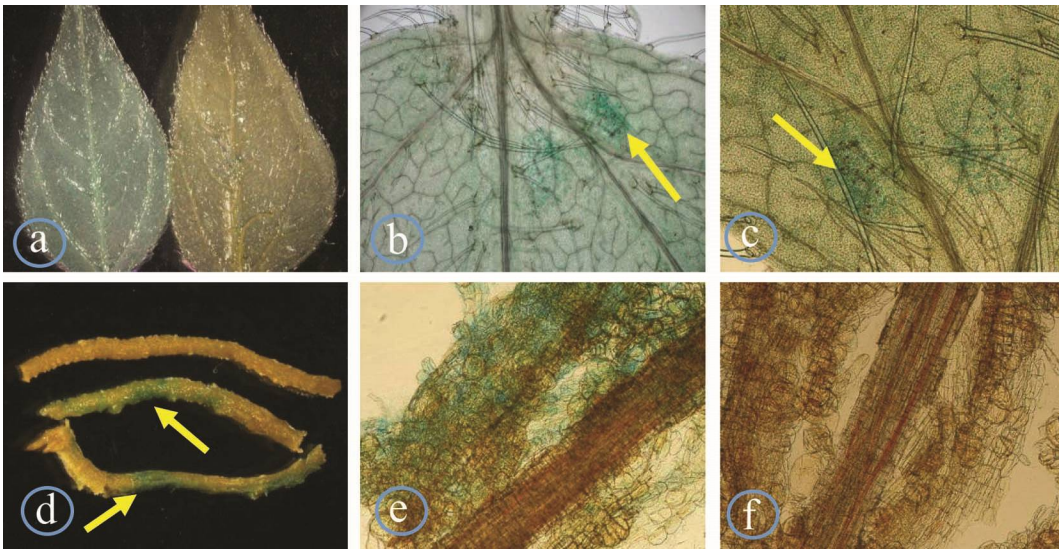

Fig. 4. Chimerical expression of *gus* gene in leaf and root tissue of the successfully transformed plants. (a) Stereomicroscopic view of the transformed leaf with control one, (b, c) light microscopic view of the regions showing GUS positive expression, (d) GUS positive expression in the transformed roots, control root showing negative GUS expression, (e) macerated cells of root tissue from transformed plant showing GUS positive response, (f) macerated cells of root tissue from control plant showing GUS negative response.

Table 1. Impact of the optical density of the *Agrobacterium* culture towards genetic transformation efficiency.

Optical density (OD_{600})	Av. GUS positive explants
0.4	2.67 ± 0.58
0.5	5.30 ± 1.53
0.6	6.67 ± 1.15
0.7	11.00 ± 1.00
0.8	8.67 ± 1.15
0.9	8.33 ± 1.52
1.0	7.33 ± 1.15

The genetic transformation efficacy also depends on the incubation period of the explants in *Agrobacterium* suspension, but long-time incubation leads to browning of the cells and the tissues become necrotic (Keshamma et al. 2008). The impact of incubation period was found to have a direct impact over the transformation efficiency. It was observed that, the transformation efficiency increases with the duration of incubation of the explants in the bacterial suspension (Table 2). But long-time exposure to the bacterial suspension caused softening of the cells and breaking during handling of the explants

that led to reduced capacity of further growth of the plants. Considering all these facts, an incubation period of 60 min was found suitable in the current study. Though there is no report of in planta genetic transformation in mungbean various tissue culture-based protocols reported various incubation period to be most efficient, for instance 45 min for cotyledon attached with embryonal axis (CAEA) (Islam and Islam 2010), 30 min for cotyledonary node explants (Sahoo et al. 2016), 15 min for double cotyledonary node explants (DCN) (Yadav et al. 2012). It is also worth to mention that the *Agrobacterium* strain and the plasmid used in these studies were different from other studies which is another reason behind achieving highest efficiency of genetic transformation for different incubation period.

Table 2. Impact of incubation period on genetic transformation efficiency.

Incubation period (min)	No. of explants assayed	No. of explants showed a positive response	Percentage of GUS positive explants
15	75	7	9.33
30	75	15	20
45	75	18	24
60	75	24	32
120	75	30	40

Addition of tobacco leaf extract in the *Agrobacterium* suspension is reported to increase T-DNA transfer efficacy (Samarajeewa et al. 2012, Rao et al. 2008) since various phenolic compounds released from wounded tissue is required for *vir* gene induction and it is found more effective than adding a single phenolic compounds such as acetosyringone (Cheng et al. 1996). Ti-plasmid bearing cells grow remarkably slower in Winans' AB medium (Winans et al. 1988) due to phosphorus limitation but this medium results in high *vir* gene expression (Morton et al. 2012). Therefore, after reaching OD_{600} at 0.7 in YMB medium the *Agrobacterium* cells were pelleted down and resuspended in Winans' AB medium. Tobacco leaf extract was added as a previously described (Rao et al. 2008) and such a way transient *gus* gene expression was increased significantly (Data not shown). In planta transformation method is relatively new and less explored than other tissue culture-based methods. Very few reports are available to date but all of them reported that vacuum infiltration treatment increases the efficacy of genetic transformation of *Agrobacterium***.** In addition to Winans' AB medium and wounded tobacco leaf extract in the present study vacuum infiltration at different durations was employed to find out how much time led to the best transformation efficiency. It was found that 2 min of vacuum infiltration followed by 60 min of incubation period in *Agrobacterium* suspension yielded highest *gus* gene expression (Table 3). In case of in

planta transformation of the de-coated half seed of black gram 2 min of vacuum infiltration is reported to enhance the genetic transformation efficacy to highest level though they have also used sonicator in combination with vacuum infiltration (Kapildev et al. 2016). Vacuum infiltration and sonication prior to incubation of explants in *Agrobacterium* suspension always enhance the T-DNA transformation efficacy (Kapildev et al. 2016, Yellisetty et al. 2015, Mayavan et al. 2013, Yadav et al. 2012).

Table 3. Effect of vacuum infiltration on genetic transformation efficiency.

Vacuum infiltration duration (min.)	No. of explants assayed	No. of explants showed a positive response	Percentage of GUS positive explants
0.0	75	21	28
0.5	75	27	36
1	75	36	48
2	75	39	52
3	75	30	40
4	75	18	24
5	75	12	16

The recalcitrant and genotype-specific nature towards *in vitro* regeneration and *Agrobacterium*-mediated genetic transformation of mungbean is an obstacle towards the genetic improvement of mungbean through genetic engineering. Moreover, due to the antibiotic selection the success rate droped drastically and in case to shoot regeneration and viable root formation *in vitro***.** Mostly the success rate varies between 1 and 2% but the rate droped further during the acclimatization stage of the *in vitro* regenerated plantlets at environmental conditions (Bhajan et al. 2019). Therefore, in planta transformation could be an alternative option to produce healthy transgenic mungbean plants. This methodology has been proved more efficient in case of *Vigna mungo* (black gram) which is very closely related to mungbean (Kapildev et al. 2016). They have reported up to 46% positively transformed plantlets.

The present study clearly indicated that *Agrobacterium*-mediated in planta genetic transformation could be a better alternative towards the successful development of transgenic mungbean plants. The efficiency of the T-DNA transformation could be enhanced by using sonication in combination with vacuum infiltration. More efficient *Agrobacterium tumefaciens* strain and Ti-plasmid could also be used to enhance the transformation efficacy.

Conclusion

It is a preliminary experiment towards *Agrobacterium*-mediated in planta genetic transformation in mungbean. It is expected that it would inspire other researchers to conduct further experiments towards transgenic mungbean plant development using this technique. A proper in planta genetic transformation protocol for mungbean would

eliminate the difficulties of *in vitro* regeneration step which is considered as the major obstacle towards the genetic engineering of mungbean.

Acknowledgement

Authors would like to thank the Bangladesh Agricultural Research Institute (BARI) for providing mungbean seeds. They also thank the University Grants Commission of Bangladesh, Agargaon, Dhaka-1207 for providing financial support through the University of Dhaka to carry out this work.

References

Baloda A and **Madanpotra S** (2017) Transformation of mungbean plants for salt and drought tolerance by introducing a gene for an osmoprotectant glycine betaine. J. Plant Stress Physiol. **3**: 5-11.

Bhajan SK, Begum S, Islam MN, Hoque MI and **Sarker RH** (2019) *In vitro* regeneration and *Agrobacterium*-mediated genetic transformation of local varieties of mungbean (*Vigna radiata* (L). Wilczek). Plant Tissue Cult. & Biotech. **29**(1): 81-97.

Cheng M, Jarret RL, Li Z, Xing A and **Demski JW** (1996) Production of fertile transgenic peanut (*Arachis hypogaea* L.) plants using *Agrobacterium tumefaciens*. Plant Cell Rep. **15**: 653-657.

Dewir YH, Murthy HN, Ammar MH, Alghamdi SS, Al-Suhaibani NA, Alsadon AA and **Paek KY** (2016) *In vitro* rooting of leguminous plants: Difficulties, alternatives, and strategies for improvement. Hortic. Environ. Biotechnol. **57**(4): 311-322.

Faruq A and **Islam M** (2010) Field performance of nine mungbean varieties against three important diseases. Bangladesh J. Agric. and Environ. **6**(1):77-84.

Himabindu Y, Reddy MC and **Chandrasekhar T** (2014) *In vitro* regeneration of green gram (*Vigna radiata* (L.) Wilczek) cultivar Vamban-2 using cotyledonary nodes. CIBTech J. Biotechnol. **3**(4): 11-15.

Hoque M, Zahan MM and **Sarker R** (2007) *In vitro* plant regeneration in mungbean (*Vigna radiata* (L.) Wilczek). Plant Tissue Cult. & Biotech. **17**(2): 209-216.

Islam M and **Islam K** (2010) *Agrobacterium*-mediated genetic transformation of mungbean (*Vigna radiata* (L.) Wilczek). Plant Tissue Cult. & Biotech. **20**(2): 233-236.

Jaiwal PK, Kumari R, Ignacimuthu S, Potrykus I and **Sautter C** (2001) *Agrobacterium tumefaciens*-mediated genetic transformation of mungbean (*Vigna radiata* L. Wilczek) - A recalcitrant grain legume. Plant Sci. **161**: 239-247.

Jefferson RA, Kavanagh TA and **Bevan MW** (1987) GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. **6**(13): 3901-3907.

Kapildev G, Chinnathambi A, Sivanandhan G, Rajesh M, Vasudevan V, Mayavan S, Arun M, Jeyaraj M, Alharbi SA and **Selvaraj N** (2016) High-efficient *Agrobacterium*-mediated in planta transformation in black gram (*Vigna mungo* (L.) Hepper). Acta Physiol. Plant. **38**: 205.

Keshamma E, Rohini S, Rao K, Madhusudhan B and **Kumar MU** (2008) Tissue culture-independent in planta transformation strategy: An *Agrobacterium tumefaciens*-mediated gene transfer method to overcome recalcitrance in cotton (*Gossypium hirsutum* L.). J. Cotton Sci. **12**(3): 264-272.

Khatun M, Haque M, Islam S and **Nasiruddin K** (2008) *In vitro* regeneration of mungbean (*Vigna radiata* L.) from different explants. Prog. Agricul. **19**: 13-19.

Kumar S, Kalita A, Srivastava R and **Sahoo L** (2017) Co-expression of *Arabidopsis* NHX1 and bar improves the tolerance to salinity, oxidative stress, and herbicide in transgenic mungbean. Fron. Plant Sci.**8**:1896.

Mayavan S, Subramanyam K, Arun M, Rajesh M, Dev GK, Sivanandhan G, Jaganath B, Manickavasagam M, Selvaraj N and **Ganapathi A** (2013) *Agrobacterium tumefaciens*-mediated in planta seed transformation strategy in sugarcane. Plant Cell Rep. **32**: 1557-1574.

Mayavan S, Subramanyam K, Jaganath B, Sathish D, Manickavasagam M and **Ganapathi A** (2015) *Agrobacterium*-mediated in planta genetic transformation of sugarcane setts. Plant Cell Rep. **34**: 1835-1848.

Mohanty I and **Sagare D** (2015) *In vitro* regeneration system in green gram (*Vigna radiata* L., Wilczek, cv. Sujata): a recalcitrant legume crop. Res. J. Agricul. Sci. **6**(1): 64-67.

Morton ER and **Fuqua C** (2012) Laboratory maintenance of *Agrobacterium*. Curr. Protoc. Microbiol. **24**(1): 3D.1.1-3D.1.6.

Niazian M, Noori SS, Galuszka P and **Mortazavian SMM** (2017) Tissue culture-based *Agrobacterium*-mediated and in planta transformation methods. Czech J. Genet. Plant Breed. **53**: 133-143.

Rao KS, Sreevathsa R, Sharma PD, Keshamma E and **Kumar UM** (2008) In planta transformation of pigeon pea : A method to overcome recalcitrancy of the crop to regeneration *in vitro*. Physiol. Mol. Biol. Plants. **14**(4): 321-328

Sahoo DP, Kumar S, Mishra S, Kobayashi Y, Panda SK and **Sahoo L** (2016) Enhanced salinity tolerance in transgenic mungbean overexpressing *Arabidopsis* antiporter (NHX1) gene. Mol. Breeding. **36**: 144.

Saini R, Singh RP and **Jaiwal PK** (2007) *Agrobacterium tumefaciens* mediated transfer of *Phaseolus vulgaris* α-amylase inhibitor-1 gene into mungbean *Vigna radiata* (L.) Wilczek using bar as selectable marker. Plant Cell Rep. **26**: 187-198.

Samarajeewa D, Kottearachchi N and **Jayawickrama C** (2012) Transformation of tomato (*Lycopersicon esculentum*) by *Agrobacterium tumefaciens* using tobacco (*Nicotiana tabacum*) leaf extract. J. Food & Agril. **2**(2): 1-5.

Schafleitner R, Nair RM, Rathore A, Wang YW, Lin CY, Chu SH, Lin PY, Chang JC and **Ebert AW** (2015) The AVRDC - The world vegetable center mungbean (*Vigna radiata*) core and mini core collections. BMC Genomics **16**: 344.

Tazeen S and **Mirza B** (2004) Factors affecting *Agrobacterium tumefaciens* mediated genetic transformation of *Vigna radiata* (L.) Wilczek. Pak. J. Bot. **36**(4): 887-896.

Vats S, Solanki P and **Alam A** (2014) Efficient *in vitro* regeneration of *Vigna radiata* (L.) Wilczek. Researcher **6**(1): 12-15.

Vijayan S and **Kirti PB** (2012) Mungbean plants expressing BjNPR1 exhibit enhanced resistance against the seedling rot pathogen, *Rhizoctonia solani*. Transgenic Res. **21**(1): 193-200.

Vir R, Lakhanpaul S, Malik S, Umdale S and **Bhat KV** (2016) Utilization of germplasm for the genetic improvement of mungbean [*Vigna radiata* (L.) Wilczek]: The constraints and the opportunities. *In:* Gene pool diversity and crop improvement: Volume 1, Rajpal VR, Rao SR & Raina SN (eds). Springer International Publishing, Cham. pp. 367-391.

Winans SC, Kerstetter RA and **Nester EW**(1988) Transcriptional regulation of the virA and virG genes of *Agrobacterium tumefaciens*. J. Bacteriol. **170**(9): 4047-4054.

Yadav S, Sreenu P, Maheswari M, Vanaja M and **Venkateswarlu B** (2010) Efficient shoot regeneration from double cotyledonary node explants of green gram [*Vigna radiata* (L.) Wilczek]. Indian J. Biotechnol. **9:** 403-407.

Yadav SK, Katikala S, Yellisetty V, Kannepalle A, Narayana JL, Maddi V, Mandapaka M, Shanker AK, Bandi V and **Bharadwaja KP** (2012) Optimization of *Agrobacterium* mediated genetic transformation of cotyledonary node explants of *Vigna radiata*. Springer Plus **1**: 59.

Yellisetty V, Reddy L and **Mandapaka M** (2015) In planta transformation of sorghum (*Sorghum bicolor* (L.) Moench) using TPS1 gene for enhancing tolerance to abiotic stresses. J. Genet. **94**: 425-434.

(Manuscript received on 24 September, 2019; revised on 21 November, 2019)