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ORIGINAL ARTICLE

Tissue-specific and stress-inducible promoters establish their suitability for containment of foreign gene(s) expression in transgenic potatoes

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Abstract

The present study was conducted to determine efficiency of green tissue-specific (pRCA) and stress-inducible promoters (pRD29A) to express *E. coli* beta-glucuronidase (*gusA*) gene in transgenic potatoes compared with constitutive promoter (35S CaMV). The promoter fragments were isolated from their original source and cloned upstream to *gusA* in pCAMBIA-1301 binary vector to develop plant expression constructs, i.e., pRCA-pCAMBIA and pRD29A-pCAMBIA. *Agrobacterium* strain GV2260 harboring recombinant plasmids were used to infect leaf discs and internodal explant of Lady Olympia cultivar. GUS histochemical analysis was performed at different stages to determine GUS activity in transgenic plants. To determine activity of stress-inducible promoter (pRD29A), transgenic plants were exposed to heat, drought and combination of both heat and drought stress. The real time (RT-qPCR) and GUS florimetric assays revealed that pRD29A promoter gets more activated under drought, heat and combination of both stresses. GUS expression levels were more than 10 folds high with pRD29A promoter compared to control. Likewise, the reduced transcripts levels of *gusA* gene under control of pRCA promoter were found in tuber/roots of transgenic plants compared to 35S promoter. GUS florimetric assays also showed decreased or no GUS expression in tubers. In conclusion, the results encourage the appropriate use of promoters to drive the expression of foreign gene(s) for the development of potato lines tolerant to biotic and abiotic stress while minimizing the risks of transgenic technology in potatoes.

Keywords Promoter efficiency · Potato · Transgene development · Targeted expression

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important cultivated plants in the world in terms of its uses as both industrial raw material and direct food. Potato has the potential to solve the insufficient problems of food resources for the increasing population because of its richness in nutrient composition (Alisdair et al. 2001). Besides that, eight species of Solanum genus are suitable for consumption as human food; however, the most widely known and produced species is *Solanum tuberosum* (Rowe 1993).

The commercialized transgenic crops express insect or herbicide resistant gene(s) under the control of 35S cauliflower mosaic virus that is constitutively expressed in all cell types and at all developmental stages of a plant (Sunilkumar et al. 2002; Amarasinghe et al. 2006; Ahmed et al. 2017). In fact, it is one of the most preferred promoters in plant biotechnology, since its discovery (Somssich 2019). However, the literature suggests that constitutive expression of a foreign gene in plants can also result in increased metabolic burden in plants, posing potential threats of resistance development against Bt toxins or herbicides (Anayol et al. 2016; Tabashnik et al. 2017; Hussain et al. 2019). The concerns about food safety of genetically modified plants have also been raised (Conner et al. 2003). Therefore, confining expression of a foreign gene (s) in specific plant tissues or activation under a particular stress can be considered significant for the development of value added crops.

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The modern technologies have opened new vistas in isolation of promoters tailored to answer specific questions in research or create new transgenic crops and products. The availability of broad spectrum promoters with the ability to regulate the spatio-temporal patterns of transgenes can increase the successful application of transgenic technology (reviewed in Potenza et al. 2004). Besides that, field of bioinformatics had led to computational analysis of primary structure and functional of a single promoter, defining enhancer function and their relationship with other motif in the genome (Reviewed in Bakhsh et al. 2011).

The targeted and confined expression of transgene(s) in specific or non-edible parts of the crop is significant and can lead to more public acceptance being less worrisome in nature (Ahmed et al. 2017). The different types of the promoters have already been isolated and characterized in this regard. Inducible promoters allow gene to be expressed when the plant is exposed to any biotic or abiotic stress; alcohol, steroid chemicals or physical factors such as light and temperature (Zhu et al. 2010).

Numerous tissue-specific promoters have been identified in plants including those involved in photosynthesis process or from seed storage genes (Mithra et al. 2017). The use of green tissue-specific promoter is ideal in crop like potato, where tubers are the edible parts. pRCA is the organ-specific and light-regulated promoter of gene encoding ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase (RCA) in potatoes (Qu et al. 2011). Inducible promoters are responsible for the expression of associate gene (s) in response to physical, chemical and environmental cues. pRD29A promoter is cis-acting element of Arabidopsis gene (RD29A) involved in responsiveness to drought, low-temperature, or high-salt stress as established earlier by Msanne et al. (2011) in Arabidopsis.

The present study was conducted to compare the efficiency of three promoters in transgenic potatoes. The promoter fragments were amplified from their sources and fused with *gusA* in pCAMBIA1301 and further transferred to potato. The results showed efficient GUS expression under pRCA and pRD29A promoter establishing their suitability to develop transgenic potatoes with trait of economic importance.

Materials and methods

Development of expression vectors

The stress-inducible (pRD29A, accession no. AY973635.1) and green tissue-specific (pRCA, accession no. HQ259068.1) promoter fragments were amplified from *Arabidopsis thaliana* and *Solanum tuberosum* cv. Lady Olympia genomic DNA sequences, respectively, using specific primers with overhangs of *Kpn*I and *Nco*I restriction enzymes. All modifications were made in binary vector pCAMBIA-1301 already available in our plant transformation laboratory. The both promoter fragments were cloned in pre-digested pCAMBIA with *Kpn*I and *Nco*I restriction enzymes upstream to *gusA* gene (interrupted by an intronic sequence to deduce expression from eukaryotic cells). The empty vector with 35S promoter upstream to *gusA* gene was used as mock in all experiments to compare the efficiency of promoters. The developed constructs were maintained in JM109 at first, and were further electroporated to *Agrobacterium* strain GV2260 using Gene Pulser Xcell™ Electroporation Systems (Cat. No. 1652660). All DNA manipulations were performed according to the standard protocols (Sambrook and Russell 2001).

Agrobacterium-mediated potato transformation

Lady Olympia potato cultivar was used as plant material in the present study. To propagate experimental explants, tubers sprouts were subjected to surface sterilization and were multiplied by monthly subculture of single-node stem explant on basal MS medium (Murashige and Skoog 1962). The potato plantlets were then maintained in a growth room with a 16/8 h light/dark photoperiod and temperature 25 ± 2 °C. The leaf and internodal explants were excised from in vitro cultured plantlets and subjected to *Agrobacterium*-mediated transformation following protocol as described by Beaujean et al. (1998) with some modification adopted by Bakhsh (2020). The regeneration media consisted of BA 2 mg/L, NAA 0.2 mg/l, Trans zeatin 2 mg/l and GA3 0.1 mg/l). During all regeneration experiments, an optimized concentration (5 mg/l) of hygromycin was used to screen transformed cells. The regenerating shoots with a length of 2–3 cm were excised and transferred to magenta boxes. The data of hygromycin resistant calli and average number of shoots were calculated after 6–7 weeks after the transformation process. To determine the transformation efficiency, all plantlets were used in PCR-based screening of construct integration into plant genome. The MS salts, sucrose, plant agar and plant growth regulators used in present study were purchased from Duchefa Biochemie. Duocid (Pfizer, Istanbul, Turkey) with ingredients of ampicillin + sulbactam (1 g + 500 mg) were also added to regeneration selection medium at concentration of 300 mg/L to eliminate excessive growth of *Agrobacterium*.

Evaluation of transgenic plants

The primary transformants were screened for the integration and expression of *gusA* gene under control of different promoters. The genomic DNA was extracted using Thermoscientific GeneJET Plant Genomic DNA Purification Kit

(Cat No. K0792). PCR assays were conducted using gene specific primers to amplify introduced gene. All PCR reactions were performed in a total reaction mixture volume of 20 µl containing 1 × reaction buffer, 50 ng of DNA template, 1.5 mM $MgCl_2$, 1 mM of each of the dNTPs, 0.5 µM of each primer and one unit of Taq DNA polymerase. The primers sequences, annealing temperature and product size have been provided in Supplementary Table 1. The plasmid DNA was used as positive control, whereas DNA isolated from untransformed plants was used as negative control. Besides that, the transformants were also subjected to PCR assay with c*hvA* gene to assess whether the primary transformants are contaminated with *Agrobacterium*. Following PCR assays, the amplified DNA fragments were electrophoresed on 1.0% agarose gel and visualized by ethidium bromide staining under ultraviolet (UV) light.

Analysis of promoter's efficiency

Two sets of experiments were performed to assess the efficiency of promoters in driving expression of *gusA* gene. For stress-inducible promoter, PCR positive in vitro cultured plantlets expressing *gusA* gene under the control of pRD29A promoter were subjected to heat and drought stress in separate as well as combined stress. The control plants (transformed with mock vector containing 35S promoter to drive *gusA* expression) was also used as comparison. The experiments were conducted in three biological repeats under four different growth conditions (Supplementary Table 2). The temperature of growth chamber was adjusted to 35 °C day and night for 2 days for heat stress. Polyethylene Glycol (PEG) 6000 (Product No: P0805.5000, Duchefa Biochemie) was used to induce drought stress. In vessels (GA-7), 50 ml MS0 medium was supplemented with 20% PEG [(optimized concentration in our earlier experiments modified from Pino et al. (2013) and Gopal and Iwama (2007)]. As we were interested to assess the efficiency of pRCA promoter in tubers of transgenic plants; therefore, the other experiment was conducted in green house. The transformants expressing *gusA* gene under the control of pRCA were shifted to 75% peat and 25% perlite along with control plants (transformed with mock vector containing 35S promoter). The samples were collected from leaf and root/ tubers.

GUS histochemical assays were performed to test the functionality of *gusA* gene under the control of 35S, pRD29A and pRCA promoters. The assay was conducted as described by Jefferson et al. (1987). To accomplish the task, GUS buffer containing 10 mg/L X-Gluc, 10 mM EDTA, 100 mM NaH_2PO_4, 0.1% Triton X-100 and 50% methanol (*Sigma-Aldrich* Chemicals Co., Merck) was prepared. The pH of the buffer was maintained at 8.0. The putative transgenic plants in both experiments were incubated in GUS buffer for 4–6 h. Furthermore, samples were destained by adding 70% ethanol to 2 mL Eppendorf tubes. Later on, image was taken under a computer-dependent (Leica M165C) microscope.

Real time PCR assays were conducted to investigate the transcripts levels of *gusA* gene under the control of different promoters in both experiments. For total RNA extraction, AMRESCO RiboZol™ RNA Extraction Reagent was used. cDNA was synthesized using Fermentas cDNA synthesis kit. RT-qPCR content included total Mix (2 ×) (Qiagen), F Primer (0.2 µM), R Primer (0.2 µM), RNase-free water. RT-qPCR temperature cycle was set up as 95 °C for 15 min, 40 cycles of 95 °C for 10 s, 58 °C for 15 s, 72 °C for 20 s and melting curve analysis was performed by incubation at 99 °C to 70 °C with a transition rate of 1.0 °C/min. For normalization, elongation factor 1-α (*ef1α*) was selected as reference gene for the purpose of quantifying the expression of genes as earlier used by Nicot et al. (2005). Each reaction was set up with three replicates. The threshold values of samples in target gene expression analysis were analyzed by software of Rotor-Gene Q (Qiagen) RT-PCR instrument. According to the results, the standard deviations of Ct values of the samples were calculated using Microsoft Excel program and the expression level of the genes was determined according to the 2-ΔΔCt proportional calculation method (Livak and Schmittgen 2001).

The fluorogenic assays were conducted to estimate the expression of GUS protein in both experiments to understand the efficiency of promoters under study as described by Bottino (2018). In both experiments, the transgenic plants were subjected to total protein isolation. Both leaves and root/tubers were collected from transgenic plants for the purpose of total protein isolation to determine efficiency of pRCA promoter along with the control. According to the growing conditions from transgenic plants, samples were collected in liquid nitrogen as leaf or root-tuber. Assay buffer was prepared by adding 1 mM MUG in the extraction buffer (17.6 mg of 4-Methylumbelliferyl-β-D-glucuronide (MUG) was added in 50 ml of extraction buffer). Taking 50 µl of the extracted proteins, 0.5 ml of assay buffer was added on it and incubated at 37 °C (3 replicates were prepared from each transgenic plant). The reaction was stopped by adding 0.2 M Na2CO3 stop buffer incubated at + 37 °C for 1 hour, 3 hours, and overnight. The image was taken by examining the results of the stopped reaction under UV lights.

Statistical analysis

The data of GUS protein activity and gene expression analysis results was analyzed using Statistix 8.1 program Statistix 8.1 (Analytical Software 2005). Significance of variance was determined after the one-way ANOVA ($p < 0.05$) followed by analysis at 5% LSD (least significant difference) method as multiple comparisons (post-hoc).

Results

The cloning of pRD29A and pRCA fragment DNA sequences upstream to *gusA* gene in pCAMBIA-1301 was achieved successfully to compare their efficiency with constitutive promoter (35S). Following confirmation by standard

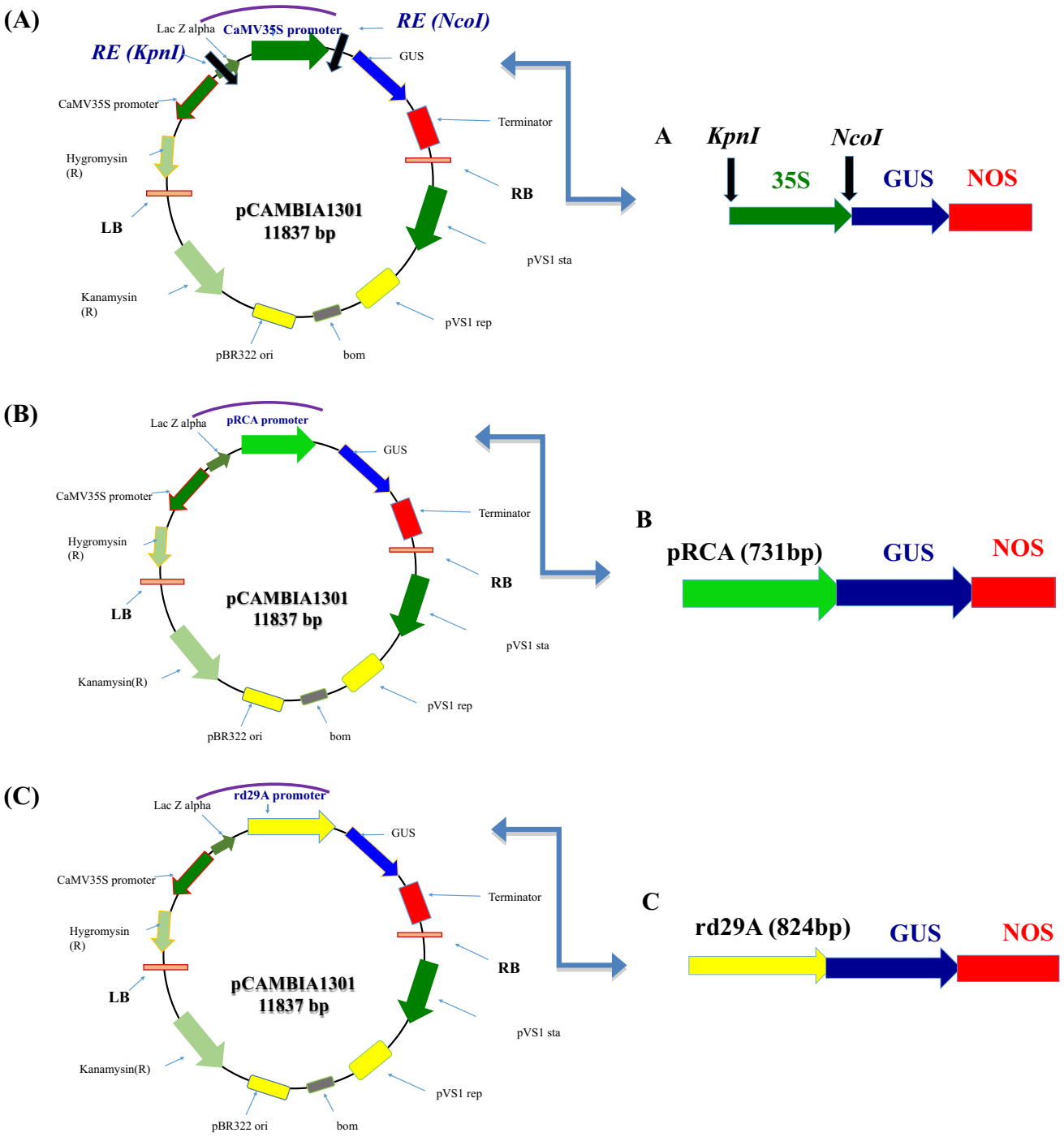


Fig. 1 Schematic images of different expression vectors developed by cloning promoters (pRCA and rd29A) in pCAMBIA-1301. **a** Original pCAMBIA1301 vector used as mock, **b** pRCA promoter was cloned upstream to *gusA* gene and named as pRCA-pCAMBIA **c** pRD29A promoter was cloned upstream to *gusA* gene and was named as pRD29A-Pcambia, respectively. The vector contains *Hygromycin phosphotransferase* (*hptII*) to encode resistance against Hygromycin that was used a plant selectable marker, whereas it contained Kanamycin for bacterial selection

molecular analyses, the recombinant plasmids were names as pRD29A-pCAMBIA, pRCA-Pcambia, as shown in Fig. 1.

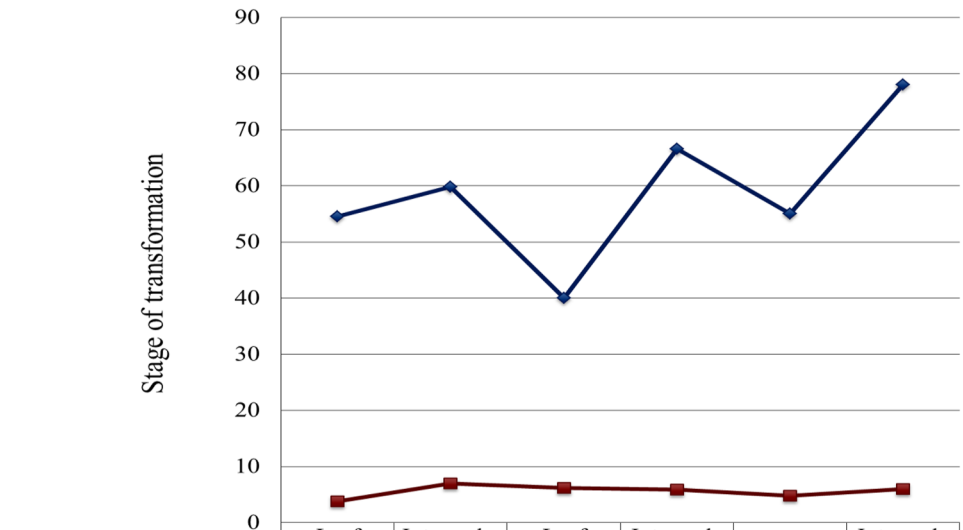
Using optimized *Agrobacterium*-mediated transformation protocol as described by Bakhsh (2020), leaf and internodes of Lady Olympia were used as explants for genetic transformation. As shown in Fig. 2, callus induction from both explants varied with different constructs. When we compared leaf and internode explants, induction of calli (60–78%) was higher in internodal explants compared to leaf (40–55%) although it varied between different constructs. Likewise, the average number of shoot in internodal explants (5.8–6.9) was also higher compared to leaf explants (3.8–6.18), whereas leaf explants transformed with pRD29A-pCAMBIA construct showed encouraging response compared to other constructs. The regenerated shoots of certain size were transferred in larger magenta boxes and were allowed rooting. All regenerated plants showed 100% rooting on a medium supplemented with optimized concentration of hygromycin.

PCR reactions showed the proper integration of T-DNA within host genomes using different primers (data shown only with *gusA* gene). PCR positive plants were selected for further studies (Fig. 3). No any amplification was observed in non-transgenic control plants. The results of *chv* gene showed absence of any *Agrobacterium* contamination. Only PCR positive plants were subjected to stress incubation assays to evaluate the efficiency of tissue-specific (pRCA) and stress-inducible promoter (pRD29A) in comparison with constitutive promoter (35S).

Histochemical GUS analysis is a cost effective and fast method to screen primary transformants. The primary transformants subjected to heat and drought stress experiment exhibited robust GUS activity (Fig. 4). Transgenic plants driving expression of *gusA* gene under the control of green tissue-specific *pRCA* promoter also showed high GUS activity in leaves when observed under microscope. While very less or no GUS activity was recorded in tubers (Fig. 4f) compared to the tubers of transgenic plants transformed with 35S-pCAMBIA (Fig. 4e). No any GUS activity was detected in non-transgenic control plants. According to histochemical GUS and PCR analysis, total transformation efficiency was determined as 0.98% in Lady Olympia potato variety.

The plantlets expressing *gusA* gene under the control of pRD29A promoter were cultured under drought, heat and both drought + heat stress. The quantitative real time data showed the increased accumulated transcript levels of *gusA* indicating approximately 10 times in drought and 15 times more in high temperature (heat stress) compared to control indicating robust activity of stress-inducible promoter pRD29A. Interestingly, transcript levels of *gusA* increased more, where both stresses (drought and high temperature in combination) were applied especially in plant No. 8 (Fig. 5b). According to the qRT-PCR data, it was observed that the expression of the *gusA* gene under the control of pRCA in transgenic potatoes increased 4–5 times more in leaves compared to tubers. No any *gusA* expression was observed in tubers of Plant No. 7 and very less in Plant No 8, where *gusA* gene was driven by pRCA promoter. Comparing with pRCA promoter, 35S transgenic potatoes showed 5 folds more expression of *gusA* gene in leaves (Fig. 6b).

To further confirm the efficacy of pRD29A and pRCA promoters, GUS protein was estimated using GUS fluorogenic assay as described by Bottino (2018). The GUS protein activity was completely absent in non-transgenic control



	Leaf pRCA	Internode pRCA	Leaf pRD29A	Internode pRD29A	Leaf 35S	Internode 35S
Callus formation (%)	54,5	59,8461	40	66,615	55	78
No. of shoots per explant	3,833	6,923	6,1818	5,8461	4,75	6

Fig. 2 Summary of transformation experiments in Lady Olympia. Callus formation (%) was calculated out of total leaf and internodal explants used in study. The shoot formation shows average number of shoots per explants (calli) in all experiments. The regenerated plants showed 100% rooting in media supplemented with hygromycin. pRCA, pRD29A and 35S here show the construct as pRCA-pCAMBIA, pRD29A-pCAMBIA and 35S-pCAMBIA, respectively

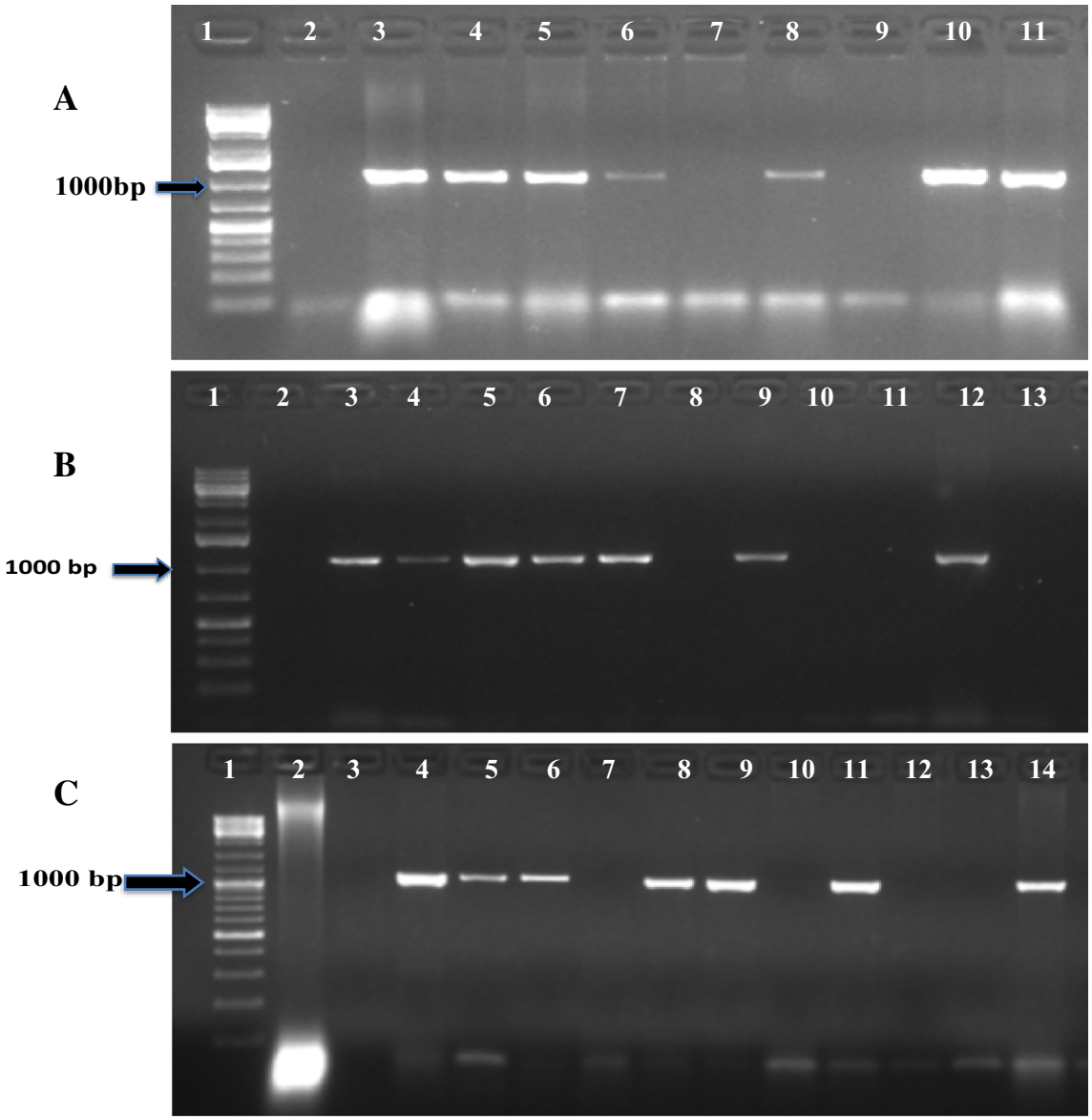


Fig. 3 PCR assays to detect gusA gene in transgenic plants **a** Amplification of *gusA* gene in plants transformed with pRD29A-pCAMBIA, Lane 1: 1 Kb plus DNA ladder (Thermo), Lane 2, non-transgenic control, Lane 3, plasmid control, Lane 4–11: primary transformants, **b** amplification of *gusA* gene in plants transformed with pRCA-pCAMBIA, Lane 1: 1 Kb plus DNA ladder (Thermo), Lane 2: non-transgenic control, Lane 3: plasmid control, Lane 4–13: primary transformants **c** confirmation of plants transformed with mock (35S-pCAMBIA), Lane 1: DNA ladder Mix (Thermo), Lane 2–3: non-transgenic control, Lane 4: plasmid control, Lane 5–14: primary transformants

potato plant (Fig. 7a), whereas pRD29A plant in control conditions showed very little activity (Fig. 7a). Overall, GUS protein activity in plants transformed with pRD29A-pCAMBIA increased under drought and heat stress in all tested plants establishing the activity of promoter under abiotic stress conditions. Plant No. 8 showed highest GUS protein under combination of heat and drought stress compared to other plants. Whereas GUS protein in the leaves of transgenic plants transformed with mock (35S promoter) and pRCA-pCAMBIA were found high (Fig. 7b). The mock transgenic plants showed robust GUS activity in root/tuber, whereas no any activity was found in tubers of plants carrying *gusA* gene under pRCA revealing the confinement of gene expression to green part of the plants. From the results of GUS fluorogenic analysis, we transferred the data to the number using color picker program according to the brightness of the samples incubated at 37 °C. According to data, the reduced or no GUS protein activity was detected in control, pRCA root/tuber and pRD29A transgenic potatoes samples grown in optimum conditions. The level of GUS

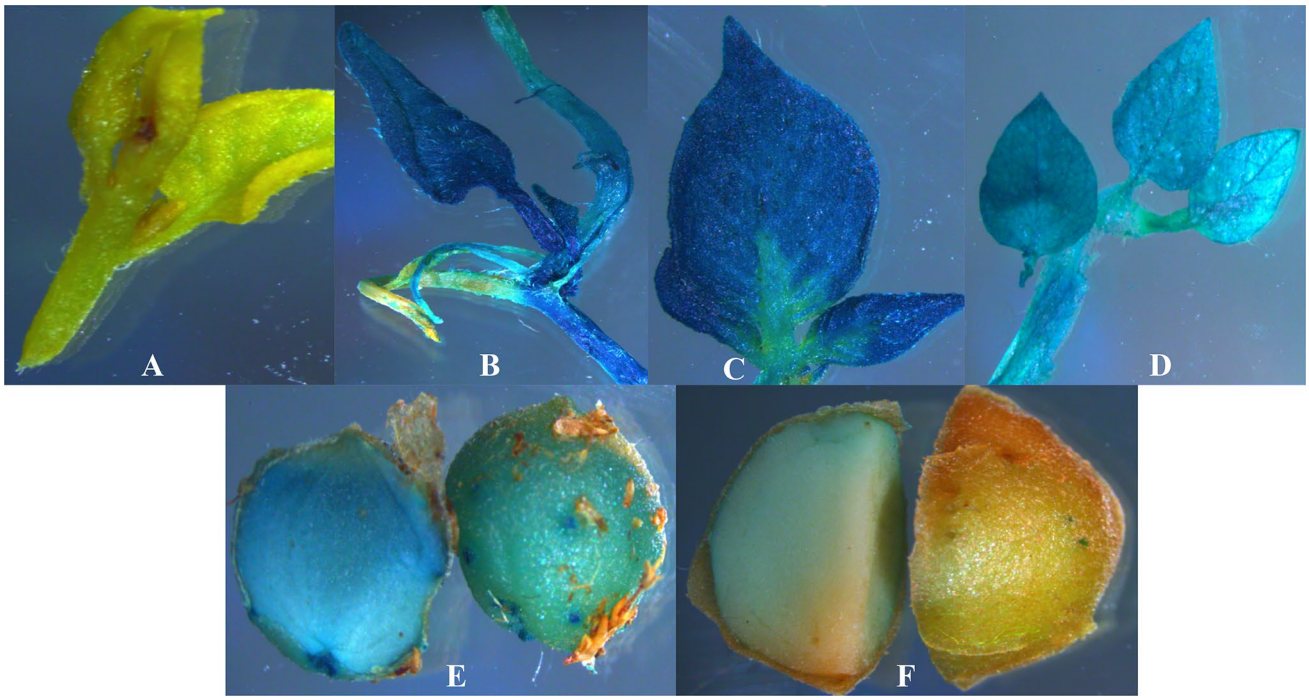


Fig. 4 Histochemical GUS analysis of transgenic potato plants expressing *gusA* gene under control of 35S, pRD29A and pRCA promoters, **a** Non-transgenic shoot, **b** transgenic plant expressing *gusA* gene under the control of 35S promoter, **c** transgenic plant expressing *gusA* gene under the control of pRD29A promoter, **d** transgenic plant expressing *gusA* gene under the control of pRCA promoter, **e** tuber of transgenic plants transformed with *gusA* gene under the control of 35S promoter, **f** tuber of transgenic plants transformed with *gusA* gene under the control of pRCA promoter

activity varied among different samples as revealed from the data (Fig. 7v).

Discussion

The current developments in genomics have assisted plant biotechnologists to identify new plant promoters to drive the expression of targeted genes spatio-temporally in crop plants (Porto et al. 2014). The knowledge about the choice and efficiency of a promoter is handy for researchers especially using new genome editing techniques like RNA interference and Crispr-Cas-9. The commercialized crop express insect or herbicide resistant gene(s) under the control of 35S CaMV; a widely used constitutive promoter (Ho et al. 1999; Bakhsh et al. 2016). There are different concerns about the use of virus based promoters in genetically modified crops (Ho et al. 1999; Podevin and Du Jardin 2012; Khabbazi et al. 2018) that provides impetus for the use of plant origin promoters in next generation genetically modified (GM) or non GM crops. Considering importance of promoters, the present study was conducted to understand the efficiency of two plant origin promoters, i.e., stress-inducible promoter RD29A and green tissue-specific promoter pRCA to drive the expression of a reporter gene (*gusA*) in transgenic potatoes in comparison with widely used 35S promoter.

To obtain transgenic potatoes, we proceeded with *Agrobacterium*-mediated transformation of Lady Olympia potato cultivar using protocol as described by Bakhsh (2020). The overall transformation efficiency in present study was recorded as 0.98% that is less when compared to earlier report of potato transformation (Veale et al. 2012; Hameed et al. 2017; Ahmed et al. 2017; Bakhsh 2020). That might be attributed to different factors, such as the type of vector, *Agrobacterium* strain, explant type, and varietal genetic background (Bakhsh et al. 2014; Heidari Japelaghi et al. 2018; Dönmez et al. 2019). The transformation efficiency was calculated according to the PCR positive plants out of total explants used and detection of GUS activity as described previous studies (Ahmed et al. 2018; Bakhsh et al. 2020). Few of plants that survived on hygromycin selection pressure in regeneration selection media could not show amplification of *gusA* gene in PCR assays; were discarded (Fig. 3). The selective agents are incorporated in regeneration selection medium during in vitro culture for selecting preferentially transformed cells by the introduction of gene (s) within T-DNA that encode resistance to antibiotic or herbicide resistance (Dandekar and Fisk 2005). Although hygromycin offers good selection system after kanamycin

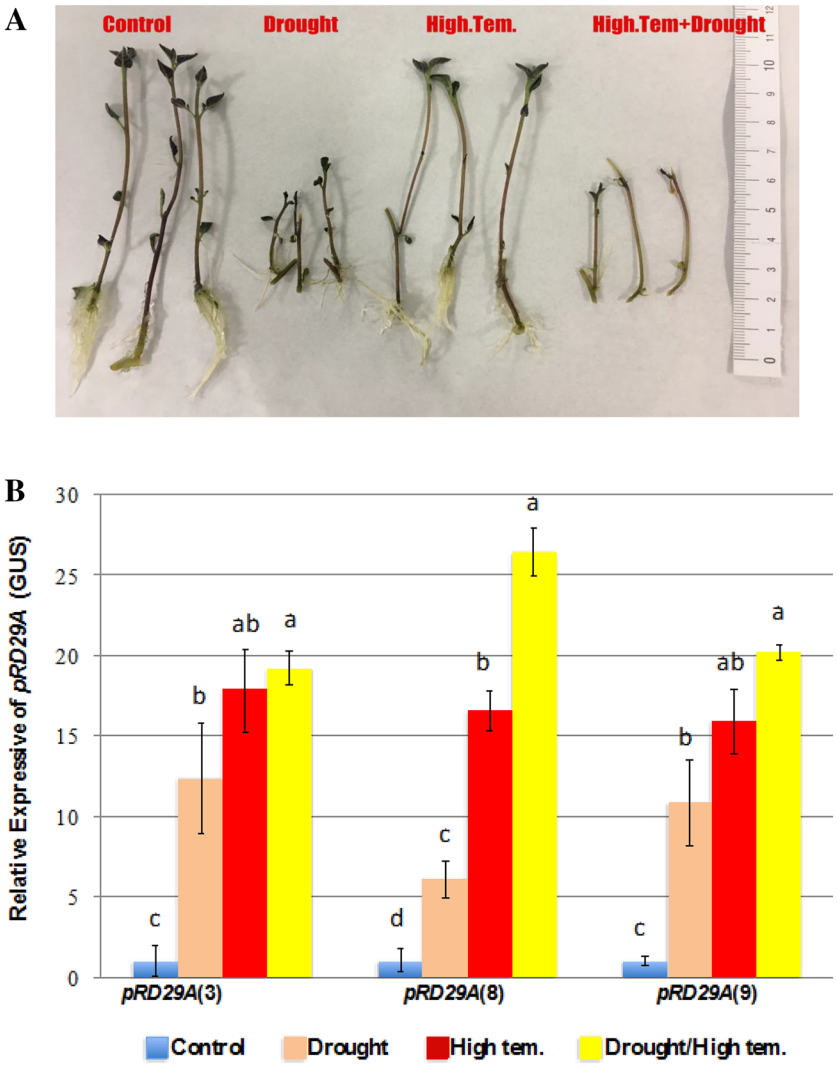


Fig. 5 Transgenic plants expressing *gusA* gene under control of pRD29A promoter were subjected to heat and drought stress separately and in combination (**a**), Coefficients of *gusA* transcript levels determined by real time PCR in transgenic plants transformed with pRD29A-pCAMBIA relative to the internal standard. For normalization of the data, EF1α was used and the expression levels were expressed as relative fold change (**b**)

during regeneration process; escapes were recorded in present study contrary to the results obtained by Zuraida et al (2013) who described hygromycin as effective selectable marker in rice to inhibit growth and development of non-transformed embryogenic calli and somatic embryos. Although it might be due to the difference of species as we optimized different concentration of hygromycin in potatoes.

The plants confirmed by PCR and histochemical assays were subjected to different analysis to evaluate the efficiency of promoters. The drought (20%PEG) and heat stress (35 °C) stresses in separate and in combination were applied to transgenic plants expressing *gusA* gene under control of stress-inducible promoter. GUS histochemical analysis of stressed plants showed that pRD29A promoter gets activated under stress and leads to the indication of GUS activity comparable to 35S constitutive promoter (Fig. 4c). Real time analysis of these plants under stress showed accumulated transcript levels of *gusA* gene indicating high gene expression under stress condition driven by pRD29A promoter in tested transgenic plants, with maximum expression in Plant No. 8 under combination of heat and drought stress (Fig. 5).

GUS gene reporter system is an invaluable tool for studying gene expression in plant related research. In present study, we followed the protocol of determining GUS activity using GUS fluorogenic assay as described by Bottino (2018). When assayed by GUS fluorogenic assays, again the plants under stress showed high GUS protein expression (Fig. 7a–c) indicating that the promoter is quite suitable for transgenic studies to drive the expression of gene(s) encoding traits of economic importance. These results are in agreement with earlier studies by Behnam et al. (2007) and Pinhero et al. (2011) that showed the activity of pRD29A promoter in Arabidopsis and potato under low temperature. However, in the present study, we were interested to determine the activity of this promoter under high temperature and drought conditions keeping in view the important of potato crop in Central Anatolian

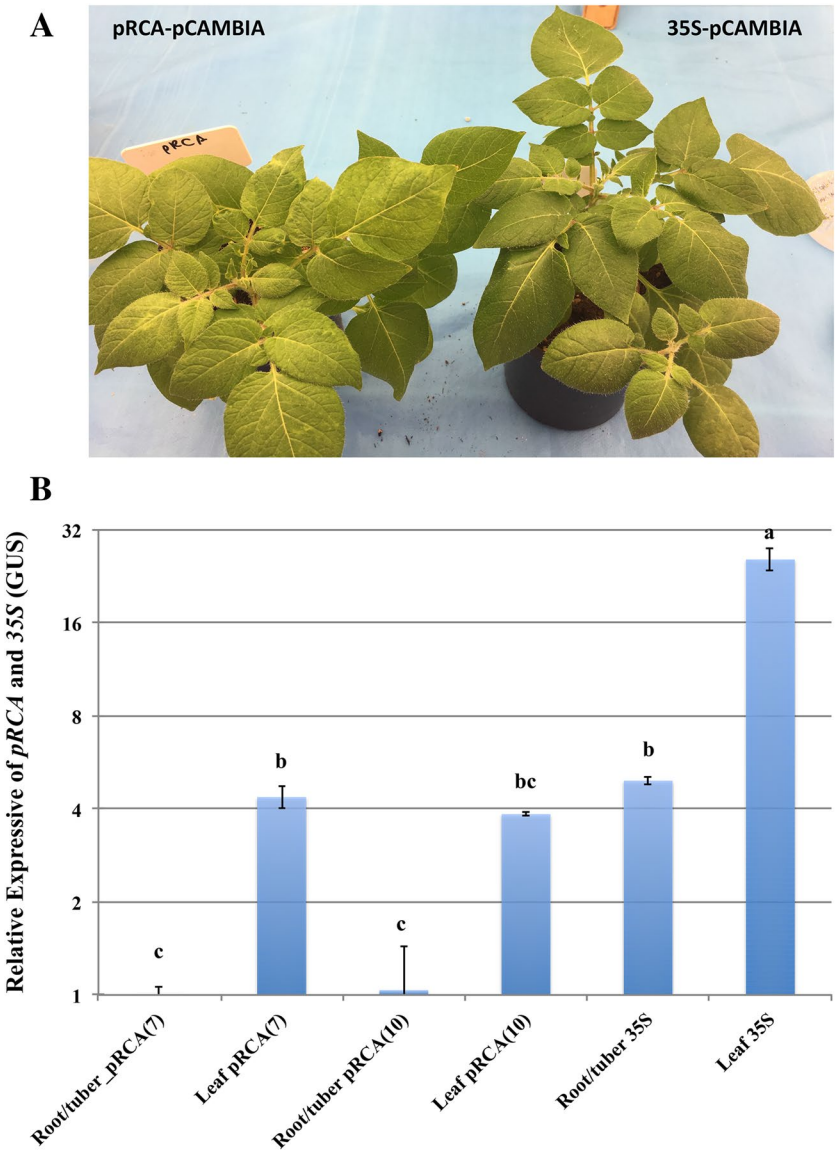
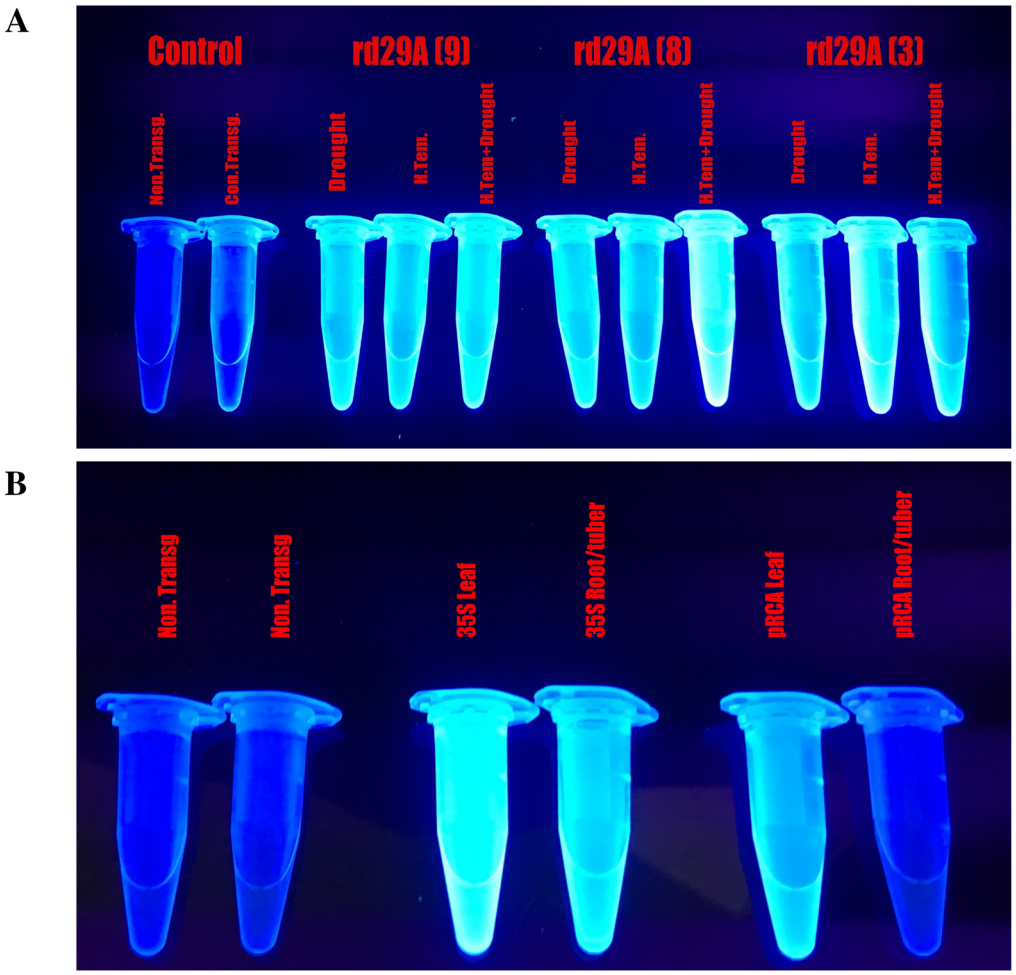


Fig. 6 Transgenic plant expressing *gusA* gene under control of pRCA promoter along with control (35S) (**a**), Coefficients of *gusA* transcript levels determined by real time PCR in transgenic plants transformed with pRCA-pCAMBIA relative to the internal standard. For normalization of the data, EF1α was used and the expression levels were expressed as relative fold change. The letters indicate the statistical difference of GUS gene expression ($p \leq 0.05$) (**b**)

region, Turkey that contributes 60% to national potato production (Çalışkan et al. 2010).

Transgenic plants with expression of *gusA* driven by pRCA promoter showed high GUS activity in leaves, again comparable to the control (35S); however, no any GUS activity was detected in tuber or roots of transgenic plants (Fig. 4) showing the organ and light specific activation of introduced gene under control of pRCA. The results of RT-qPCR further showed the least expression of *gusA* gene in tubers and roots of transgenic plants (Fig. 6), although leaf showed high GUS expression in all three tested plants, lesser than 35S promoter. These results are in agreement with earlier reports in crops like sweet potato (Tanabe et al. (2015) and cotton (Bakhsh et al. 2012), where high transgene expression was recorded using green tissue-specific promoters with low or no expression in seed (Özcan et al. 1993) and tubers (Rahamkulov 2019). Likewise GUS protein was not detected in root and tubers of transgenic plants with pRCA promoter indicating the suitability of promoter in transgenic technology to contain transgene expression to green parts of the plant that can be ideal for many crops like potato and corn. It is important to mention here, 35S promoter driven GUS protein was high in leaves and tubers of control plants (Fig. 7). The light and organ-specific promoters of genes encoding small and large subunit of Ribulose-1,5-bisphosphate carboxylase/oxygenase have been characterized and utilized in earlier studies (reviewed in Potenza et al. 2004; Bakhsh et al. 2011). After establishing pRCA promoter role in inducing tissue-specific specific expression of *gusA* gene, we used this promoter to drive expression of dsRNAs of insect molting-associated Ecdysone receptor gene in potatoes against Colorado potato beetle (CPB) and confirmed


A
Control
rd29A (9)
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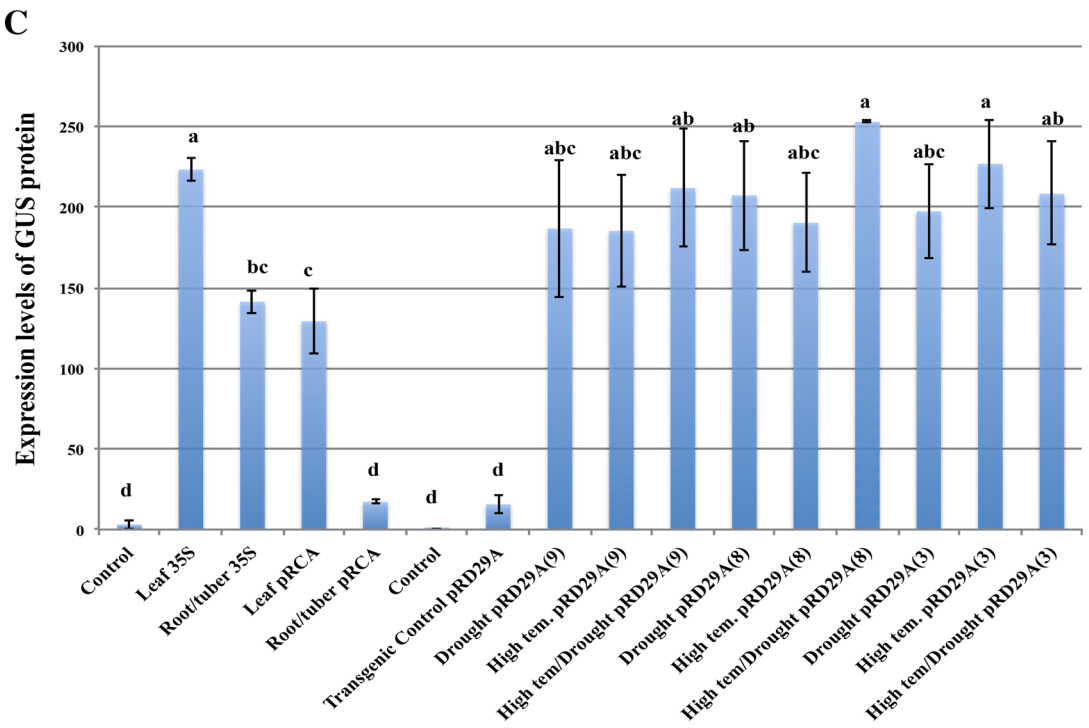
35S Leaf
35S Root/tuber
pRCA Leaf
pRCA Root/tuber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Expression levels of GUS protein
300
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Control
Leaf 35S
Root/tuber 35S
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Root/tuber pRCA
Control
Transgenic Control pRD29A
Drought pRD29A(9)
High tem. pRD29A(9)
High tem/Drought pRD29A(9)
Drought pRD29A(8)
High tem. pRD29A(8)
High tem/Drought pRD29A(8)
Drought pRD29A(3)
High tem. pRD29A(3)
High tem/Drought pRD29A(3)

◂**Fig. 7** GUS fluorogenic assays of transgenic plants expressing *gusA* gene under pRD29A promoter (**a**), primary transformants expressing *gusA* gene under the control of pRCA promoter (**b**). GUS proteins data from the results of GUS fluorogenic analysis with the color picker program. The letters indicate the statistical difference of GUS protein activity ($p \leq 0.05$)

the confinement of dsRNAs in green part of the plants with increased mortality of CPB (Unpublished data).

Since commercialization, advantages and disadvantages of genetically modified crops are being discussed. To minimize the biosafety concerns and to increase the acceptance of transgenic crops in public, the use of tissue-specific and stress-inducible promoters can be consummated to drive the expression of foreign gene(s) in crops.

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Author contributions The data presented in the manuscript is MS thesis work of IR who completed his studies under the supervision of AB.

Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

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