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Over expression of stress responsive *Pennisetum glaucum 47* helicase (*PG47*) improves stress tolerance in groundnut (*Arachis hypogaea* L)

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ABSTRACT

RNA helicases function as molecular motors that rearrange RNA secondary structure, potentially performing roles in any cellular process involving RNA metabolism in an ATP-dependent manner and play an important role in protein synthesis. *Pennisetum glaucum* 47 (*PG47*) RNA helicase overexpressed in groundnut (*Arachis hypogaea* L.) cultivar GPBD-4 improved drought tolerance. The transgenics plants were confirmed for presence, expression and stable integration by Kanamycin screening, genomic DNA PCR, RT-PCR and Southern analyses respectively. In T3 generation, the promising transgenic events were identified based on stress tolerance and improvement in productivity. The transgenic events showed enhanced stay-green phenotype and increased chlorophyll stability under drought stress. The transgenics also showed reduced chlorophyll retardation under NaCl, PEG and etherel-induced stress conditions. Transgenic plants showed increased yield than wild type under stress conditions. Results suggested that *PG47* RNA helicase contributing for enhanced drought-adaptive traits and improved productivity under water-limited conditions.

Key words: Drought, Groundnut, Stress tolerance, Yield.

Abbreviation: CLT- cellular level tolerance, PDH45- Pea DNA helicase 45, TDM-Total dry matter.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an economically important oil and protein rich crop, whose seeds contain about 40 to 50% oil and 26 to 28% protein that has a significant impact in tropical and sub-tropical regions of Asia, Africa, North and South America. It is being cultivated on over 25.2million ha worldwide with a total production of 41.2 million tons with an average yield of 1.67 tons/ha. India is the second largest producer of groundnut accounting 8 million tons from 6 million ha (FAOSTAT, 2014) which needs to be increased up to 14.8 million tons by 2020 to meet the growing demand.

Drought and salt are major abiotic stresses that adversely affect crop productivity. Thus, identification of factors that confer resistance to these stresses would pave way to increasing agricultural productivity (Zhu 2002). Transgenic approaches have been attempted to improve tolerance to abiotic stresses. Many regulatory and functional genes that bring about cellular level tolerance are fairly well elucidated and the relevance of several candidate genes is well established. Helicases are the molecular motors belonging to the DEAD box protein family that catalyze the unwinding of nucleic acids in an ATP- dependent manner. Stress induced RNA helicases essential for survival during stress in plants (Tuteja and Tuteja, 2004).

RNA secondary structure rearrangements are catalyzed predominantly by members of the two protein families either RNA helicases or RNA-binding proteins (RBPs). RNA helicases refer to enzymes that use energy derived from the hydrolysis of a nucleotide triphosphate to unwind double stranded RNAs (De la Cruz, 1999). The majority of RNA helicases belong to the superfamily 2 (SF2), consists of three subfamilies, termed DEAD, DEAH and DExH/D, based on variations within a common DEAD (Asp-Glu-Ala- Asp) motif (Tanner and Linder 2001).The range of enzymatic activities, and thus potential physiological activities, exhibited by RNA helicases has expanded to include rearrangement of ribonucleoprotein (RNP) complexes via the removal or 'clearing' of protein from RNA and the combination of both RNA unwinding and RNA annealing to promote RNA-strand exchange, potentially through a branch migration mechanism. RNA helicases have been implicated in every step of RNA metabolism, including nuclear transcription, pre-mRNA splicing, ribosome biogenesis, nucleocytoplasmic transport, translation, RNA decay, RNAi, RNA editing and organellar gene expression (Rocak and Linder 2004). The amino-acid sequence of *PG47* (*Pennisetum glaucum 47*) shows high homology with *PDH45* (Pea DNA helicase 45), translation initiation factor eIF- 4A from tobacco, *Arabidopsis thaliana*, maize and wheat (Pham

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et al. 2000). Overexpression of *PDH45* under constitutive promoter conferred salt tolerance in tobacco and rice with improved yield (Amin *et al*. 2012) and sugarcane (Sruthy *et al*. 2015). In the present study the emphasis is to overexpress RNA helicase, *PG47* to improve drought tolerance in groundnut. The stress-responsive RNA helicase *PG47* was overexpressed under RbcS promoter to improve cellular level tolerance (CLT) in a trait donor groundnut. The transgenics plants showed enhanced cell membrane thermo stability and up regulation of a few stress-responsive genes leading to abiotic stress tolerance and yield improvement in peanut.

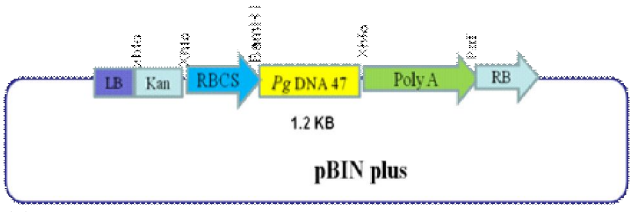
MATERIALS AND METHODS

The plasmid cloning site of *PG47* was identified by sequential digestion of PBSK vector 1.2 kb fragment was released in *XhoI/BamHI* subsequently *PG47* fragment were end filled with *EcoR V* and digested with *Bam HI & XbaI* and ligated to in a TA vector. From TA vector *PG47* fragment released using *XbaI & BamHI* and ligated to in impact vector which contains RbcS promoter. Whole cassette (promoter, gene and terminator) was released with *Asc I & PacI* enzymes and ligated in to the binary vector pBin plus (Fig.1a). The pBinplus*PG47* plasmid was mobilized to *Agrobacterium* strain LBA4404 by electroporation method and the positive clones were identified using colony PCR analysis with gene-specific primers. Insert was confirmed by digestion with *XbaI & BamH I* enzyme and also by PCR with gene specific primer and *npt II* primer.

Plant transformation and selection of transgenic plants: Seeds of groundnut (*A. hypogaea* L.) cv. GPBD-4 were obtained from the National, Seed Project (NSP), the Agricultural Research Station Dharwad, Karnataka, India. Seeds were surface sterilized according to Kiran *et al*. (2009). Cotyledonary nodes were excised aseptically from 4-5 day old seedlings and inoculated on to modified Murashige and Skoog's (MS 1962) medium containing 3% (w/v) sucrose. All the cultures were maintained at a temperature of 25±2°C under a 16/8-h (light/dark) photoperiod provided by cool white, fluorescent lamps. For plant transformation, *PG47* was driven by RbcS promoter construct was used for plant transformation *via A. tumefacience* (strain LBA4404). *Agrobacterium* was grown at 28°C in AB minimal medium supplemented with kanamycin (50 mg transgenic) and used to infect explants for 4–5 min at room temperature. Infected explants were inoculated on MS plus shoot initiation media (SIM) containing BAP (3 mgL^{-1}), NAA (1 mgL^{-1}), kanamycin (125 mgL^{-1}) and cefotaxime (250 mgL^{-1}). Once appreciable growth was seen, the explants were transferred on shoot proliferation media (SPM) containing BAP (3 mgL^{-1}), NAA (1 mgL^{-1}), kanamycin (125 mgL^{-1}) and cefotaxime (250 mgL^{-1}). Developed shoots were transferred to shoot elongation media (SEM) containing GA (1 mgL^{-1}) kanamycin (125 mgL^{-1}) and cefotaxime (250mgL^{-1}) for shoot elongation. Plantlets were then transferred to root induction media (RIM) containing IBA (0.5 mgL^{-1}), kanamycin (75 mgL^{-1}) and cefotaxime (250 mgL^{-1}) . The rooted plantlets were transplanted into pots containing soilrite and covered with plastic bags to prevent dehydration, and subsequently allowed for hardening under controlled environmental conditions. After one week, the plants were transplanted to pots containing potting mixture and allowed to grow to maturity in the transgenic containment facility.

Kanamycin germination assay: For selection of transgenic plants, germinated seeds were soaked in kanamycin (400 ppm) for 5 h and subsequently transferred to sand medium supplemented with nutrient solution, and allowed to grow for 15 days. The plants with good root growth were selected and progressed to next generation (Fig 2a).

Molecular analysis of transgenic plants: PCR Analysis: Genomic DNA of transformed and wild type plants for molecular analysis was isolated. PCR was performed with the purified genomic DNA using primers for *PG47*, marker



(a)

(b)

Fig 1: a: Schematic diagram of constructed vector map for the over expression of *PG47*.**b:** Plant transformation: i Shoot proliferation of putative transgenic peanut plantlets on selection medium containing BAP (3 mg L^{-1}), NAA (1mg L^{-1}), kanamycin (125 mgL^{-1}) and cefotaxime (250 mg L^{-1}): ii Shoot elongation of putative transgenic peanut plantlets on selection medium containing GA (1 mg L^{-1}), kanamycin (125 mg L^{-1}) and cefotaxime (250 mg L^{-1}):iii Rooting of putative transgenic peanut plantlets on selection medium containing IBA (0.5 mg L^{-1}), kanamycin (75 mg L^{-1}) and cefotaxime (250mg L^{-1}): iv Increase in yield was observed in some of the putative transformants when compared to wild type v Representative gel showing the PCRanalysis of the putative transformants for RbcS forward and *PG47* gene-specific reverse primer. Lanes1–25Transgenics,W Wildtype, M1kbladder, P plasmid.

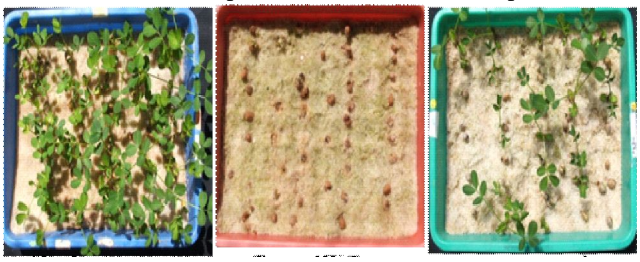
genes (*nptII*) and RbcS forward and *PG47* gene-specific reverse primer were used to amplify the *PG47* gene to avoid endogenous amplification (Table1).

To amplify the fragments in both the reactions, PCR was initiated by a hot start at 94 °C for 5 min followed by 31 cycles of 94 °C for1 min, 56°C (*PG47*),57°C (RbcS F and *PG47* R) and 58°C (*nptII*) for 45 s and 72°C for 1 min with a final extension of 10 min. The amplification was checked on 0.8 % agarose gel.

For Southern analysis, a 30 µg genomic DNA sample from promising lines and untransformed controls were digested with *Hind III* and probed with 887 bp DNA, resolved on 1% agarose gel and blotted on a *Hybond N+* (*Amersham Intermational*, Little Chalfont, Bucks, UK) membrane. The blot was probed with the 32P-radio labeled *PG47*cDNA (Sambrook *et al*. 1989).

Total RNA was isolated from leaves by following phenol chloroform method. Total RNA was reverse transcribed to generate cDNA by using Revert Aid Reverse Transcriptase (MMLV-RT; MBI Fermentas, Hanover, MD, USA) using oligo (dT) primers) following manufacturer's instructions. cDNA products were further amplified with *PG47* sense primer 5'-ACTTCAGTCCGTGAGGACC-3' and antisense primer 5'-CACGGGATGAACCAGACCTGA -3' to detect transcription of the *PG47*gene. An ELF depolymerizing factor (sense primer: 5'- GCAGCATCA GGTATGGCAGT-3' and antisense primer: 5'- TTCCAT CAAGTTCCCCTCTT-3') was amplified with all cDNA products to check their integrity. Negative control reactions without reverse transcriptase were performed for each sample to test DNA contamination (Sambrook *et al*. 1989).

Physiological analysis for abiotic stress tolerance: The stress response of the selected transgenics under in vitro conditions in the leaf disks was analyzed by imposing PEG, NaCl, Ethrel and Methyl viologen stress. Leaves of plants grown under control conditions in containment were used for the experiment. Fully expanded third leaf of wild type and the selected transgenic lines was sampled and leaf disks of 1 cm diameter were made and incubated in 15 %PEG solution in petri plates for 72 h. For the NaCl, leaf disc are placed on a 350 mM NaCl for 24hrs and for the Methyl viologen leaf disks were placed on 10µl of Methyl viologen solution in petri plates for 72h. At the end of the stress period, the stress effects on total chlorophyll content and the integrity of the cell membrane were estimated.



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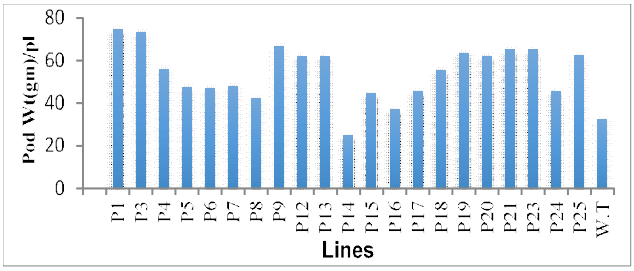

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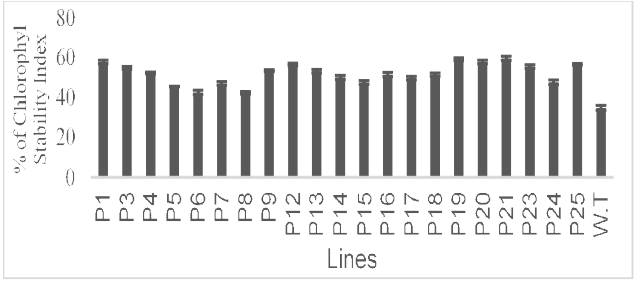

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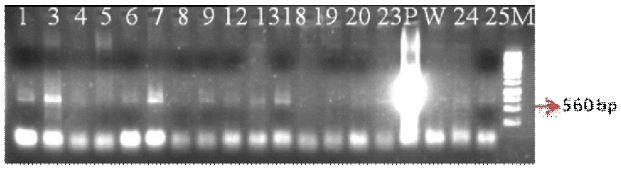

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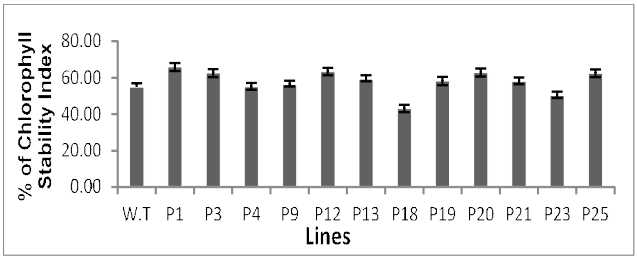


(e)

Fig 2: Molecular, Physiological and yield analysis of T1 and T_2 transgenic plants. **a:**Kanamycin germination (400 ppm) screening of putative T1 transgenic groundnut seedlings. **b:**T_1 transgenic plants showing Chlorophyll Stability Index in NaCl. **c:**Pod weight of T1generation transgenic plants.**d :** PCR analysis of the T2 transformants for *PG47*gene specific primers. Lanes 12 Transgenics, W Wild type, M1 kbladder, P plasmid .**e:**T2transgenic plants showing Chlorophyll stability Index (CSI) in NaCl.

Table 1: Primers used in the PCR analysis.

Primer name	Forward sequence (5'-3')	Reverse sequence (5'-3')
RbcS promoter F-*PG47*R	*CAGCCTCTCCTTACCACGAA*	*TCACACCCAGGTAGTCACCA*
PG47 F - *PG47* R	*AACTTCAGTCCGTGAGGACC*	*CACGGGATGAACCAGACCTGA*
nptII F - nptII R	*GAGGCTATTCGGCTATGACTG*	*ATCGCGAGGGGCGATACCGTA*

Table 2: Yield analysis of the transgenic lines under control moderate and sever stress conditions.

Lines	Control condition	Moderate stress	Sever stress	Average of yield (g/pl)
P1	72.3	46.8	18.3	45.80
P3	65.4	29.5	12.4	35.77
P4	65.3	35.5	10.1	36.97
P9	58.9	37.7	18.5	38.37
P13	62.1	42.6	11.4	38.70
P19	57.3	32.4	12.3	34.00
P20	55.4	39.3	16.4	37.03
P25	60.2	35.4	15.9	37.17
Mean±SE	58.36±4.11	35.18±2.81	13.79±.09	23.2
GPBD-4	38.4±5.3	22.4±3.2	8.8±1.2	
S. D	12.35	8.2	2.71	

In this experiment mean ± SE and SD of three independent replicates.

Estimation of chlorophyll stability index: The effect of stress on chlorophyll stability index was estimated by quantifying the chlorophyll content after alleviation of the PEG and NaCl stress following the standard procedure (Arnon 1949) in fresh leaf samples of transgenics and wild type plants.

Electrolyte leakage assay: Leaves from 3-week-old transgenic and wild type plants were excised and floated in petri plates containing NaCl (300 mM) for 24 h and PEG 15% solution for 48 h. the leaf disks (50 mg) of the wild type and transformants were incubated in 10 ml of deionized water for 1 h. Initial electric conductivity (EC) was taken and analyzed for membrane damage by electrolyte leakage assay using conductivity meter (Elico-India, CM183, EC-TDS analyzer) (Govind *et al*. 2009).

Methyl Viologen assay: Wild type and transgenic leaf discs (100mg) were floated in 10µM of Methyl Viologen solution for 8 hours and 16 hours under dark conditions. Membrane leakage and Chlorophyll Stability was estimated using conductance TDS meter (Systronics Pvt. Ltd) and expressed as micro Siemens (µS) (Hiscox and Israelstam *et al*. 1979).

Etherel induced senescence: The resistance of transgenics to ethylene-induced senescence using ethereal (2-chloro ethyl phosphonic acid) was studied. Lethal concentration of etherel was standardized at 2,000 ppm. Leaf disks from 75 days-old plants grown under control conditions were exposed to ethereal solution (2,000 ppm) in dark for 72 h and analyzed for total chlorophyll content (Hiscox and Israelstam *et al*.,1979).

Rate of water loss: Third fully expanded leaf from the cotyledonary branches was collected by cutting at the joint of leaf petiole with the stem and immediately stored in polythene bags to reduced water loss. Initial fresh weight of the leaf was recorded and the leaf with four leaf lets was spread over a filter paper on the laboratory table at a temperature of 25±2°*C* and light intensity of 250 µ moles/ m^2/s^1 (Arnon 1949). The fresh weight of the leaf was recorded at hourly interval up to 6 hours and the loss in weight in relation to original weight of the leaf was computed.

Rate of water loss = (FW0-FW1)/FW0*100

FW0 =Initial fresh weight

FW1 = weight at hourly interval

Specific leaf area (SLA): The fully expanded leaf of each selected plants were collected and leaf area was measured using T area meter/leaf meter. Then the leaves were dried in oven until constant weight was obtained. Then weight of each leaf was taken separately. Then Specific leaf area was calculated.

SLA (cm^2/g) = (LA/DW)

Where, LA is leaf area in cm^2 and DW is dry weight in grams.

Analysis of the transgenic lines on productivity under stress: An experiment was conducted to analyze the effect of moisture stress and productivity in the selected *PG47* transgenics grown in soil under containment conditions. In this experiment plants were exposed to two moisture stress regimes. In one set of plants moderate stress was imposed by withholding water for 15days from 75 DAS. Similarly, severe stress was imposed by withholding the water for 25 days from 75 DAS from another set of plants with well watered control. After the stress period, plants were all aviated from the stress and maintained at ûeld capacity till harvest.

A comparative analysis of the transgenic events was carried out to assess various yield attributes. Yield in terms of total dry matter (TDM), pod number, and pod weight were recorded and compared with the wild type.

Data were subjected to analysis of variance (*ANOVA*). Mean values were calculated using Duncan's new multiple range test. Experiments were repeated twice, each time with 3 replicates.

RESULTS AND DISCUSSION

Many processes like osmotic adjustment, cell cycle regulation, protein turnover and removal of toxic compounds including reactive oxygen species (ROS) are considered as the major components of CT mechanism. RNA binding proteins (RBPs) are involved in maintaining mRNA stability under diverse cellular process including growth, development and stress response (Hunseung *et al*. 2013).

Transgenics in groundnut cv GPBD-4 with *PG47* gene were developed following the *Agrobacterium* mediated transformation. Cotyledonary nodes excised from 4-day-old seedlings were used to generate transgenic peanut plants by *Agrobacterium* mediated transformation.

Explants were co-cultivated with *Agrobacterium* (LBA4404) and are grown on shoot initiation medium (SIM) without any antibiotics for two days. Further, they were sub cultured into the shoot proliferation medium supplemented with MS salts, BAP and NAA (Fig.1b i). Kanamycin resistant induced shoots were transferred to shoot elongation medium (SEM) containing GA and kanamycin (Fig.1b ii). After 15 days, elongated shoots were subculture into 1/2 MS medium containing IBA plus kanamycin for rooting (RIM) (Fig.1b iii). A total of 650 explants were used for transformation, untransformed shoots in the selection medium turned necrotic and died subsequently. Healthy and well rooted plant lets were transplanted to pots in green house (Fig. 1b iv). These 55 T0 transformants were subjected to PCR analysis for the amplification of *PG47* gene specific (Fig 1b v), *nptII* gene, RbcS forward and *PG47* gene specific reveres primers (data not shown).The PCR confirmed putative transformants showed considerable variability in plant height, pod weight and TDM compared to the wild type. Based on pod weight and repeated PCR analysis, 21 lines were advanced to T1 generation for further analysis.

Analysis of T_1 and T_2 transformants: The transgenic lines were rigorously screened for kanamaycin sensitivity at 400 ppm (Fig 2a). Based on physiological (Fig 2b and 2e), yield (Fig 2c) and PCR analysis (Fig 2d) of T1 and T2 generation, eight promising plants were selected [1-1-2-2 (P1), 3-2-1-1 (P3), 4-3-2-2 (P4) 9-1-2-2 (P9), 13-3-1-2 (P13), 19-1-2-3 (P19), 20-3-1-2 (P20) and 25-1-1 (P25)] and are advanced to T3 generation. In this study transgenics in successive generation have been screened not only stable for integration based on kanamycin sensitivity and molecular characterization but also stress tolerance and productivity. The lines showed higher tolerance and higher productive were advanced to the successive generation.

Analysis of T_3 transformants: Comprehensive analysis for the stability of integration, stress response and productivity was carried out in the 8 selected T3 generation transgenic lines. One set of the plants were raised under control condition in greenhouse for molecular and physiological analysis and other subset of plants were exposed moderate and severe drought stress condition to assess the genotype and productivity PCR analysis of the T3 generation plants showed the ampliûcation of *PG47* with gene specific primer (Fig.3a) thus confirming stable integration of transgene. From the transgenic plants P1 and P3 the 560 bp ampliûed PCR product of the *PG47* gene was restrict digested with *Hind III* ennezymes that produced 302 and 158 bp expected fragment sizes (Fig. 3b), which conûrmed the authenticity of the integrated transgene.

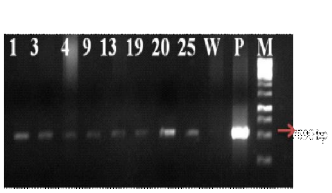
Southern analysis was carried out to assess the stable integration of the T-DNA in all the selected groundnut plants transformed with *PG47* gene. Genomic DNA was digested with *Hind III* and transferred to nylon membrane after electrophoresis and hybridized with the α^{32}P-labeled gene specific primer probe. Transgenic lines P1, P9, P13, P25 are the single copy transforments and P4 showed two copies were observed in the selected plants. The variation in the hybridization pattern among the various transgenic lines demonstrated the independent nature of the transgenic events (Fig. 3c). Further the transgenic plants were analyzed for expression of transgene. The total RNA was extracted and reverse transcribed to get cDNA from the southern positive lines. Increased level of expression of the transgene confirmed the transcript accumulation in the transgenic plants compared to wild type plants (Fig. 3d).

The DEAD box RNA helicases actively disrupt misfolded RNA structures by ATP hydrolysis, modulation of RNA structure that influences splicing, mRNA export and translation (Dunand-Sauthier *et al*. 2005). *PG47* showed 86% homology with eIF-4A and hence we hypothesize that it is involved in translation and unwinding secondary structures of mRNA. The RT-PCR data showed *PG47* helicase transcript is induced in groundnut transgenics.

Stress Response of Selected Transgenic Lines

PEG and etherel stress: The excised leaf discs of the selected transgenics lines were stressed under PEG for chlorophyll stability index and membrane integrity. The selected transgenic lines especially [P1,P13, P20, P25] demonstrated higher chlorophyll stability and lesser electrolyte leakage under PEG stress compared to the wild type (Fig.3e). Some of the transgenic plants showed less electrolyte leakage of 36.8–45.3% when compared to the wild type which showed 52% electrolyte leakage,thus indicating reduced membrane damage in transgenic under dehydration stress.

The chlorophyll retention under the induced senescence conditions was more in the lines P1, P3 and P25 which also displayed the stay-green phenotype. The plants



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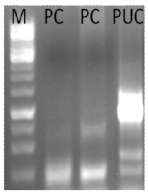

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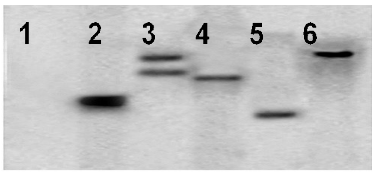

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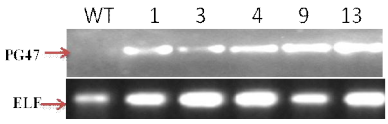

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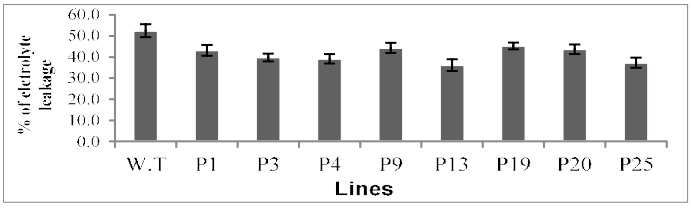

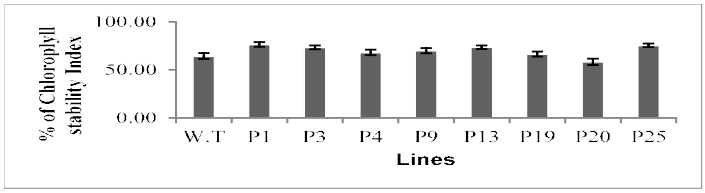

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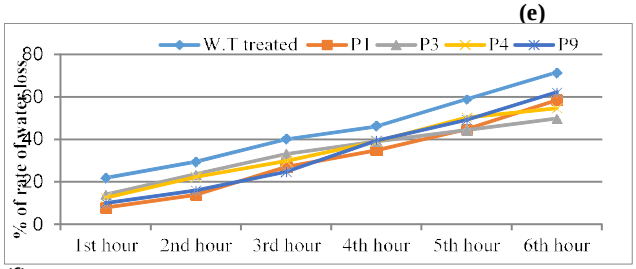

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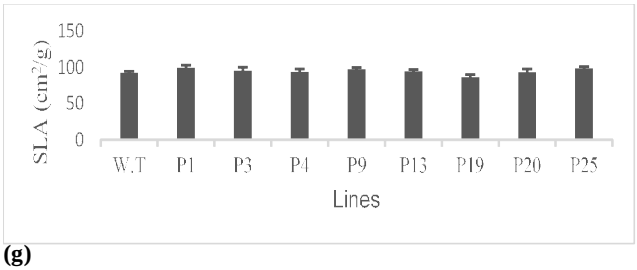

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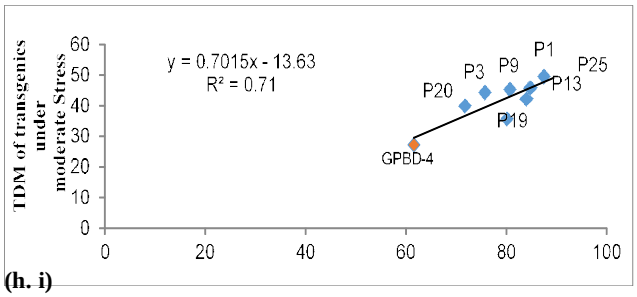
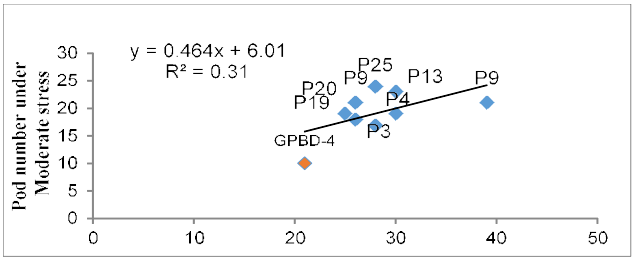


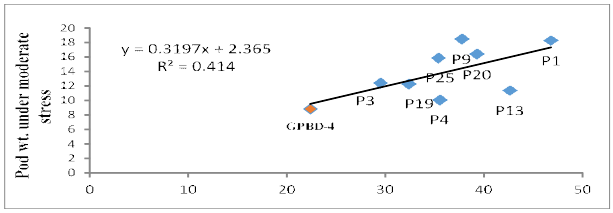
(h. i)

TDM of transgeins under severe Stress



(h. ii)

Pod number under Sever stress



(h. iii)

Pod weight under sever stress

Fig 3: Molecular, physiological and yield analysis of transgenics in T3 generation.

a: PCR analysis with gene-speciûc primers 8 Lanes of transgenics, W wild type, M 1kb ladder ,P plasmid. **b:** *PG47*gene assessed by restriction enzyme analysis of the PCR product. Lanes1–2 PCR product from the transformed plants, and Lane M marker and PUC plasmid uncut PCR product of the plasmid **c:** Genomic Southern analysis.Lane:1wildtype; 2-6 transgenics.**d:** Semi-quantitative RT-PCR analysis. Lane1–5 transgenics. ;W wildtype. **e:** PEG6000 stress on chlorophyll stability index &membrane integrity of the transgenic lines in comparison to wild type. **f:** Rate of water loss of transgenic lines in comparison to wild type **g:** Comparison of Specific leaf area of wild type and transgenic lines. **h:** Association studies between different yield attributes of the transgenics and wild type under moderate and severe water-deûcit stress. i Pod number, ii TDM, and iii Pod weight.

with reduced membrane damage under stress maintaining better membrane integrity will help in functional stability of macromolecules (Levitt 1980).

NaCl stress: Exposure of leaf disc to NaCl stress induces chlorophyll degradation leading to marked decline in photochemical efficiency. From this context, the performance of transgenic plants was assessed under NaCl stress. Leaves of the transgenic lines P1, P13, and P25 showed lesser chlorophyll degradation under NaCl compared to wildtype plants. The data indicate that transgenic leaves had higher CSI (chlorophyll stability index) under stress compared to wild type (Data not shown).

Methyl viologen stress: Leaf disc assay was carried out by imposing oxidative stress using methyl viologen. Total chlorophyll content of stressed leaf discs was analyzed and was compared with non-stressed leaf discs. There was significantly low level of total chlorophyll in wild type plants compared to transgenic plants under stress. Similarly, there was increased cell damage in wild type plants due to oxidative stress than the transgenic as evidenced by electrolyte leakage. The results revealed that the transgenics have relatively stable membrane integrity than wild type (Data not shown).

Rate of water loss (RWL): Fresh weight of the excised leaves of both transgenic lines and the wild type plants leaves was recorded at hourly intervals up to six hours. The average loss in fresh weight ranged between 40-62% in the transgenic plants whereas wild type plants showed an average of 71.2% (Fig.3f). In response to several abiotic stresses including salt (specifically Na+), dehydration, oxidative stress (methyl viologen) and water stress caused by desiccation. It indicated that there was positive correlation between regulation of transgene and stress tolerance. Similar kinds of results have been reported with *mtlD, AtDREB1A* expressing groundnut lines also (Sarkar *et al*. 2016).

Specific leaf area (SLA): There was significant difference in specific leaf area between the transgenic and wild type plant. The average specific leaf area was 92.6 cm^2/ g in *PG47* transgenics, where as it was 88.2 cm^2/ g in wild type (Fig.3g).

Improvement in the yield and productivity in the selected transgenicevents: In the second subset, to analyses the drought response, *PG47* expressing transgenic plants, and wild type plants were subjected to gradual moisture stress. Selected plants were grown and exposed to moderate and sever stress for 15 and 25 days respectively and control plants were maintained under well watered condition. Analysis of productivity of the selected transgenic events indicated signiûcant improvement of productivity (Table 2). The average yield of the transgenic plants was signiûcantly higher when compared to wild type in control treatment as well as in plants subjected to moderate and severe stress conditions. Many of the selected events *viz*.P1, P3, P4 and P13 showed signiûcant improvement in productivity ranging from 34.00 to 45.8 g/plant when compared to 27.50 g/plant in the wild type. Further, yield attributes like TDM, pod number, and pod weight were compared in the transgenic lines under control, moderate and sever stress conditions. The lines that showed improved productivity also had improved biomass over the wild type which was evident under control condition, moderate and severe stress. Further, association studies between control, moderate and severe stress showed a positive correlation among TDM, pod number and pod weight (Fig. 3h i-iii). *PG 47* transgenic was shown to enhance stay green phenotype, higher leaf water status, higher cell membrane stability, and increased biomass under stress (Table 2). Senescence is an internally programmed degeneration process induced by drought and other environmental factors (Nooden1997). The importance of stay green trait has been well elucidated in transgenics which have demonstrated delay in the onset of senescence (Rivero 2009). As shown in the earlier studies by Manjulatha *et al*. (2014) and Santhosh *et al*. (2017) transgenics express RNA helicase also showed higher chlorophyll content and a stay green phenotype under stress which in fact resulted in higher photosynthetic efficiency, even increased growth rate of plant. The productivity analysis of the eight selected events in these experiments conûrmed that expressing *PG47* showed a positive phenotype with improved productivity under stress condition. Transgenic events P1, P3, P4 and P13 are superior and promising.

In Conclusion, overexpression of *PG47* RNA helicase improves tolerance in groundnut by maintaining the RNA processing machinery under moisture stress conditions.

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