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Evaluation of Transgenic Groundnut Lines Under Water Limited Conditions

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Groundnut (*Arachis hypogaea*), an annual legume crop, is the third major oilseed of the world, and is produced in tropical and sub-tropical regions. Asia accounts for 66.5% of the world groundnut production while Africa produces only 24.7%. Poor yields of the groundnut crop are often due to abiotic constraints like drought or low soil fertility. Annual estimated losses in groundnut production, equivalent to over US$520 million, are caused by drought (Sharma and Lavanya 2002). Yield losses due to drought are highly variable in nature and depend mainly on the timing, intensity and duration of drought.

Genetic improvement for drought tolerance is crucial in many regions where agriculture depends on scarce water resources. The finding of the genes involved in the tolerance to drought and their insertion in the genetic background of agronomically preferred varieties could enhance and/or stabilize the yields under drought-prone situations. Therefore, a holistic approach integrating physiological dissection and molecular marking of the tolerance traits is needed (Subbarao et al. 1995) to understand the mechanisms underlying tolerance, and to insert these traits into agronomically desirable material. Plant survival under severe drought is an important aspect of the tolerance to drought as it contributes to ensure a minimum yield in subsistence farming.

Research in transgenic crops may offer new means to improve agriculture, particularly in dry areas, as genes specifically involved in the response to drought have been identified (Liu et al. 2000). However, a major challenge of transgenic research, besides obtaining transgenic material, is to understand the physiological expression at the plant level of the inserted genes. Transgenic groundnut lines from the parent JL 24, with enhanced survival under moisture deficit conditions, have been developed. The process included transformation of drought-responsive elements and transcription factors,

like DREB1A cDNA driven by drought-responsive promoter rd29A, which specifically interacts with the DRE inducing the expression of stress tolerance genes (Shinozaki and Yamaguchi-Shinozaki 1997). Fourteen transgenic lines of T-2 generation along with the untransformed JL 24 were evaluated. This study was conducted to: (i) assess the transpiration response of transgenic groundnut to water deficit in comparison to the control JL 24; and (ii) select a few lines with contrasting responses for further detailed studies (leaf gas exchange characteristics of transgenic material).

Methods

The dry-down experiment was conducted according to previous work using FTSW (fraction of transpirable soil water) as a covariate (Sinclair and Ludlow 1986, Ray and Sinclair 1997) and included exposure of plants to progressive drought by withholding water. The dry-down plants typically go through three stages. Stage I occurs when there is non-limiting soil water to fully supply transpiration demand. Stage II occurs when the roots no longer fully supply transpiration demand and stomatal conductance decreases to adjust transpiration to available water. Stage III occurs when stomatal conductance is at a minimum and can no longer decrease.

Twelve plants per transgenic line were grown under well-watered conditions until 19 days after sowing in glasshouse conditions with 20/12∞C day/night temperatures. Single plants were grown in 20-cm diameter pots, filled with 4.5 kg Alfisol taken from a field with low nitrogen content (8.5 mg kg^{-1}) and mixed with 4 g of single super phosphate. Seeds were inoculated with *Bradyrhizobium* NC 92 (IC 7001) (1 g L^{-1}) to ensure adequate nodulation of groundnut and 2 g of carbofuran. At 19 days after sowing, pots were saturated with water prior to exposure to water stress. The pots were sealed with polythene bags to prevent any water loss directly from the pots. The pattern of the transpiration response to soil drying and FTSW was examined on the basis of recorded daily weights of the pots. The water loss by transpiration in irrigated control pots was added back daily. No water was added to drought stressed plants. Normalized transpiration rate (NTR) was calculated to compare the transpiration of drought stressed plants to that of control and to minimize the effect of plant-to-plant variation (Ray and Sinclair 1997). Drought stressed plants were considered to have extracted all the extractable water from the pot when NTR was less than 10% of the transpiration of controls, defined as the end point of the dry-down (beginning of stage III). A plateau regression procedure using SAS (SAS Institute 1989) and NTR as a function of FTSW was applied to calculate the threshold at which the stomatal closure initially occured, ie, when transpiration began to decline. This allowed the calculation for the number of days between initial decline of transpiration and the end of the dry-down for each transgenic line (stage II).

Results

Control JL 24 started to show wilting symptoms (loss of turgor) after 21 days of stress and thereafter, severe symptoms were evident in this line. It took 27 days for control JL 24 to reach stage III (NTR<0.1). The transgenic lines showed no wilting symptom even after 21 days. Thereafter, transgenic lines started to vary in their wilting symptoms, with a few transgenic lines showing no symptoms, while lines RD 14, RD 22 and RD 25 showed reduced level of symptoms (compared to JL 24). The transgenic lines differed largely in the time (number of days) to reach the end point (Table 1). RD 14 reached the end point in 29 days, about the same time as control JL 24, while RD 4 reached the end point at 52 days. All the lines had similar growth at the time when

Table 1. Average number of days to end point of the drying cycle for groundnut transgenic lines along with control JL 24.

Line number	Number of days to end point[1]
JL 24 (control)	27 e
RD 14	29 de
RD 13	32 d
RD 25	32 d
RD 19	33 d
RD 22	34 cd
RD 12	36 c
RD 28	36 c
RD 30	39 bc
RD 20	44 b
RD 23	44 b
RD 21	45 b
RD 2	47 ab
RD 11	49 ab
RD 4	52 a

1. Means followed by the same letter have overlapping 95% confidence intervals using Duncanís multiple range tests.

drought stress was applied, so the differences in plant responses reported here are not related to plant size.

The data of NTR, FTSW and number of days to end point were subjected to average linkage cluster analysis for preparing dendrogram using Euclidean distance of NTSYSPC (Version 2.10 d). The dendrogram (Fig. 1) showed that the lines could be broadly classified into four groups at 0.6 SI (similarity index), which clearly distinguished the water-use pattern among these lines, and suggested that the transgenic lines differed in their stomatal response to water deficit.

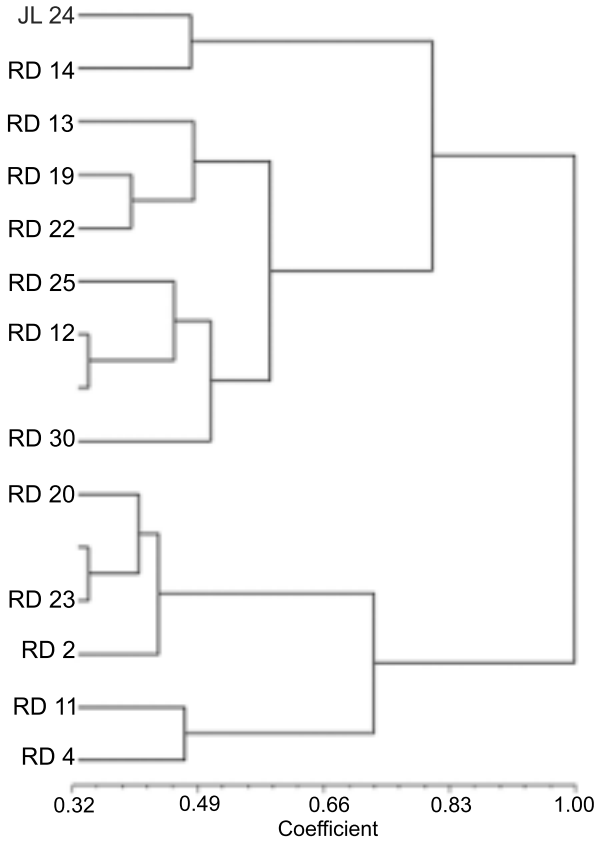

Figure 1. Dendrogram showing relative similarities in 15 transformed groundnut lines (including control JL 24) based on FTSW threshold values and the number of days to end point under water deficit conditions.

The results confirmed that drought-responsive elements inserted in the transgenic groundnut plants are linked to stomatal regulation. The fact that certain transgenic genotypes withstand drought for longer periods, and how this relates to stomatal closure needs further investigation, as there might be a scope to use transgenic plants for inducing drought tolerance. Here, we identified contrasting transgenic material to assess the physiological response of stomata under drought, with RD 4 withstanding drought for long while RD 14 was similar to control JL 24. A selection of contrasting transgenic lines from this study is being used in detailed experiments to confirm present results, and further investigate the link between the differences in stomatal closure and the transpiration efficiency.

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