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Full Length Research Paper

Simple sequence repeat (SSR)-based genetic variability among peanut genotypes different in specific leaf weight and relative water content

Surasak Boontang[1], Nathpapat Tantisuwichwong[2]*, Sanun Jogloy[1], Chutipong Akkasaeng[1], Nimitr Vorasoot[1] and Aran Patanothai[1]

[1]Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand.
[2]Department of Biology, Faculty of Sciences, Khon Kaen University, Khon Kaen 40002, Thailand.

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The objective of this study was to compare if simple sequence repeat (SSR) markers could correctly identify peanut genotypes with difference in specific leaf weight (SLW) and relative water content (RWC). Four peanut genotypes and two water regimes (FC and 1/3 available water; 1/3 AW) were arranged in factorial randomized complete block design with six replications. The data were recorded for specific leaf weight (SLW) and relative water content (RWC), and 256 SSR markers were used to detect genetic difference. 89 SSR markers could detect polymorphism among peanut genotypes (48.9%). The numbers of alleles ranged from 1 to 6 with a mean of 2.7 alleles per locus. The polymorphic information content (PIC) values varied from 0.38 to 0.75 with a mean of 0.48. The genetics relationship among peanut genotypes was estimated. KK 4 was clustered distinct from the others genotypes, whereas ICGV 98324 and ICGV 98303 were grouped in the same cluster furthest from the KK 4. The results from this study could be useful as a source of variation for development of mapping population for drought tolerance in peanut breeding program.

Key words: Drought, water regime, polymorphism, genetic relationship, SSR markers.

INTRODUCTION

Most peanut production areas in the semi-arid tropics are usually affected by drought stress caused by unpredictable rainfall and rain distribution (Wright and Nageswara Rao, 1994). Drought stress can occur at any time during crop growth, causing severe yield loss and poor seed quality. Improvement of drought tolerant peanuts is thus a sustainable means to cope with drought problem (Branch and Kvien, 1992). Most peanut breeding programs for drought tolerance conducted so far have been based on selection for pod yield *per se*. This method has slow progress because of large effect of genotype x environment interaction.

The physiological traits associated with drought tolerance have been suggested to determine drought tolerant genotypes (Songsri et al., 2008). Specific leaf weight (SLW) and relative water content (RWC) are physiological parameters related to drought tolerance (Nigam and Aruna, 2008; Nautiyal et al., 1995). Peanut genotypes with high SLW had higher transpiration efficiency (Brown et al., 1996), and this trait had low genotype x environment interaction and high heritability (Songsri et al., 2008). Specific leaf area (SLA; closely associated with

*Corresponding author. E-mail: naptan@kku.ac.th. Tel: 0066 43 342908. Fax: 0066 43 364169.

Abbreviations: SSR, Simple sequence repeat; **SLW,** specific leaf weight; **RWC,** relative water content; **DAE,** days after emergence.

SLW) also has negative relationship with stomatal conductance, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) enzyme and carbon exchange rate (CEC). Under water limited conditions, peanut genotypes with low SLA could maintain higher RWC and normal growth (Nautiyal et al., 2002). Thus, it is possible to use SLA to evaluate drought tolerance in peanut (Vasanthi et al., 2006; Upadhyaya, 2005).

DNA markers have been developed to assist selection of economically important traits. The progress of selection should be more rapid than the conventional methods because it can be conducted in early generation of segregating population with more accuracy (Stalker and Mozingo, 2001). The methods for identifying markers and traits associations are based on genotypic and phenotypic data from specific crossed population that shows a difference in the target traits and genetics level (Utami et al., 2008).

Previous studies in peanut showed that genetic variability at DNA level in this crop is very low. Many marker types such as isozyme, random amplified polymorphic DNA (RAPD), restriction fragment length polymorphic DNA (RFLP), and amplified fragment length polymorphic (AFLP) could not detect the polymorphism or had very low polymorphic among cultivated peanuts because of narrow genetic base (Halward et al., 1991,1992; Lanham and Fenneil, 1992; Lack and Stalker, 1993; Gracia et al., 1995; Stalker et al., 1995 ; Kochert et al., 1996; He and Prakash, 1997; Gimenes et al., 2002).

Hopkin et al. (1999) first developed Simple Sequence Repeats (SSRs) technique for peanut, and this technique was further used successfully to detect polymorphism in cultivated peanuts (Krishna et al., 2004; Ferguson et al., 2004; Moretzsohn et al., 2004). These markers are small arrays of tandem arranged bases (one to six) spread throughout the genomes and are abundant, informative, and co-dominant in nature. Recently, SSR markers have been recognized as useful tools in plant breeding program such as genetic diversity analysis (Cuc et al., 2008; He et al., 2005; Jiang et al., 2007; Mace et al., 2006), germplasm management (Barkley et al., 2007), genome mapping, QTL analysis and applicable for marker assisted selection (Chenault et al., 2009; Ferguson et al., 2004; Varshney et al., 2009; Yan bin et al., 2008).

To the best of our knowledge, DNA markers associated with SLW and RWC in peanut have not been previously reported. The question underlying the research project is whether SSR markers are associated with SLW and SLA or not. Therefore, peanut accessions were screened for SLW and RWC, and the genotypes with the contrasting characters were tested for polymorphism of SSR markers. The objective of this study was to verify that peanut genotypes with differences in SLW and RWC are also different in SSR markers. The information is useful for the development of mapping population for drought tolerance in peanut breeding program.

MATERIALS AND METHODS

Three peanut genotypes (ICGV 98324, ICGV 98353 and ICGV 98303) previously identified as drought tolerance from ICRISAT and further identified as high SLW and RWC in our preliminary study (Boontang et al., 2010) were used in this experiment. They were compared with KK 4 previously identified as low SLW and RWC (Akkasaeng et al., 2007; Boontang et al., 2010). SSR markers were used to determine genetic difference among these peanut genotypes with difference in SLW and RWC under well-water and water limited conditions.

Physiological measurement

The experiment was conducted at the Field Crop Research Station of KhonKaen University located in Khon Kaen province, Thailand (latitude 16° 28´ N, longtitude 102° 48´ E, 200 m above mean sea level) during November 2006 to April 2007. A 2 x 4 factorial experiment in a randomized complete block arrangement with six replications consisting of one pot per replication was undertaken under open environment in the field. Two soil moisture levels at field capacity (FC) (10.28%) and 1/3 available water (AW) (5.33%) were assigned as factor A and four peanut genotypes were assigned as factor B.

The plants were grown in cement containers with 25 cm in diameter and 70 cm in height. Each container was filled with 43.6 kg of air-dried soil (Yasothon series; loamy sand, Ocix Paleustults) to 10 cm from the top of the container. Soil was separated into four columns to create uniform bulk density. Plastic tubes were installed at three positions on the container at the heights of 15, 30 and 45 cm from the bottom for irrigation purpose. Water was then supplied to the container through the plastic tubes and on the soil surface.

The soil physical properties were 71.2% sand, 2% silt and 8.7% clay. The chemical properties included 5.58 soil pH, 0.47% organic matter and 0.02% total nitrogen, 7 ppm available phosphorus (Bray II and Molybdenum-blue method) 23.5 ppm extractable potassium and 216.5 ppm calcium.

The seeds were treated with captan (3a 4,7,7a-tetrahydro-2-[(trichloromethyl)thio] -1H-isoindole-1,3(2H)-dione) at the rate of 5 g kg^{-1} seed before planting. Four seeds were planted for each container and the seedlings were then thinned to two plants pot^{-1} at four DAE. Phosphorus fertilizer as triple superphosphate at the rate of 12.12 g P pot^{-1} and potassium fertilizer of muriate of potash (KCl) at 15.26 g K pot^{-1} were applied at four DAE. Gypsum ($CaSO_4$) at the rate of 9.58 g Ca pot^{-1} was applied at 33 DAE. Pest and disease were controlled by weekly applications of carbosulfan [2,3-dihydro-2,2-dimethyl-7-benzofuranyl (dibutylamino) thio methylcarbamate 20%, w/v, water soluble concentrate] at 2.5 l ha^{-1}, methomyl [S-methyl-N-((methylcarbamoyl)oxy) thioacetimidate 40% soluble powder] at 1.0 kg ha^{-1} and carboxin [5,6 dihydro-2-methyl-1.4 oxathiine-3 carboxanilide 75% wettable powder] at 1.68 kg ha^{-1}.

The soil moisture was uniformly maintained at FC until 4 DAE for uniform germination. After 4 DAE, water supply at 1/3 AW was withheld until the soil moisture content reached the predetermined level of 1/3 AW (5.33%) at 19 DAE, whereas water supply at FC was continued until harvest. Irrigation was done on the soil surface and through three positions on the containers where the tubes were installed. The soil moisture was maintained uniformly with no more than 1% moisture change of predetermined until 60 DAE.

In maintaining the soil moisture levels, water was added to the containers based on crop water requirement and surface evaporation as described by Doorenbos and Pruit (1992) and Singh and Russell (1981), respectively. Calculation of total crop water use for each water treatment was calculated as the sum of crop water requirement and soil evaporation. Crop water requirement was calculated using the methods described by Doorenbos and Pruit

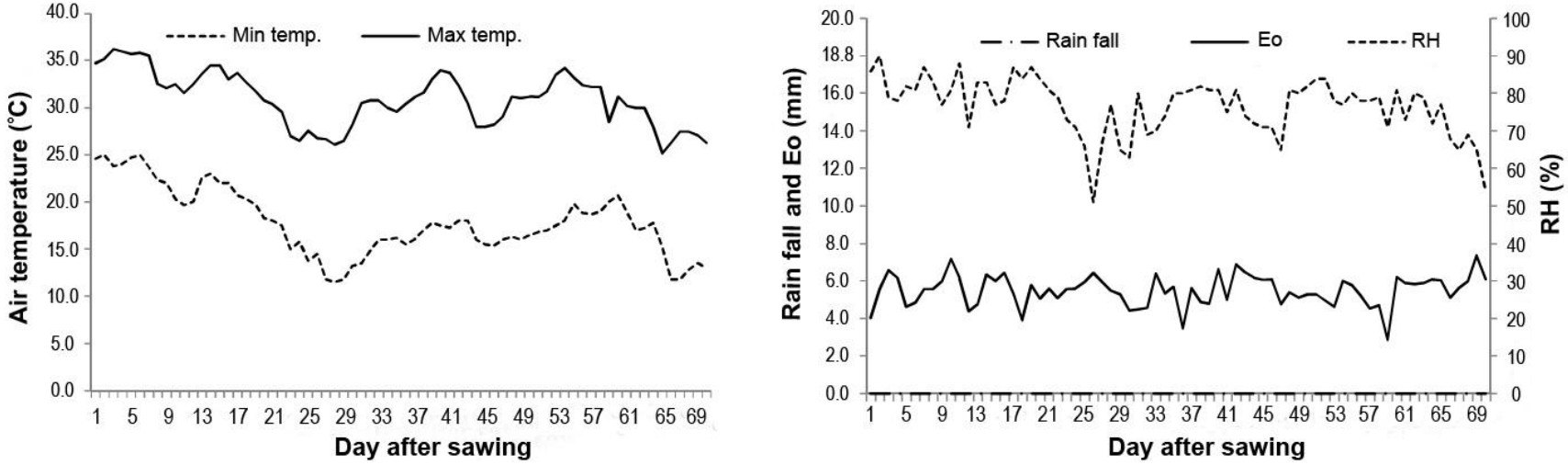

Figure 1. Maximum and minimum mean air temperature, total rainfall, evaporation (E_o) and relative humidity (RH) during growing season.

(1992):

ETcrop = ETo x Kc

Where, ETcrop is the crop water requirement (mm/day); ETo is the evapotranspiration of the reference plant calculated by using class A pan evaporation method and Kc is a crop water requirement coefficient of peanut.

The surface evaporation was calculated by using the method from Singh and Rusell (1981):

Es = β x (Eo / t)

Where, Es is the soil evaporation (mm); β is the light transmission coefficient; Eo is evaporation from class A pan (mm/day) and t is the day from last irrigation.

Weather data were obtained from the meteorological station closed to the experimental site as shown in Figure 1. Monitoring soil moisture content was observed at 40, 50 and 60 DAE by collecting the soil samples from the depths between 0t o60 cm using micro auger. The soil samples were then placed in aluminum containers and sealed with paraffin to prevent moisture loss. Wet soil samples were weighted, oven dried at 105°C for 48 h and sample dry weights were determined. Soil moisture content was calculated as:

Soil moisture = [(weight of wet soil – weight of dry soil)/ weight of dry soil] x 100

Soil moisture status showed reasonable management of soil moisture. A clear distinction among soil moisture levels was noted at 40, 50 and 60 DAE (Figure 2).

Physiological data collection

At 40, 50 and 60 DAE, fully–expended leaves were detached from the second nodes from the top of the main stems during the morning period (0900 to 1100 h.), placed in sealable plastic bags in ice bath and taken immediately to the laboratory. Leaf samples were weight and leaf fresh weight was recorded. Leaf areas were measured using an LI 3100 leaf area meter (LICOR, Lincon, USA). The leaf samples previously used for SLW measurements were placed in distilled water at 20°C for 8 h until saturated, and then saturated or turgid leaf weight was determined. The leaf samples were then dried at 80°C for 48 h and dry weight was determined. SLW was calculated as:

SLW = Leaf dry weight (g) / Leaf area (m^2)

The RWC was calculated as:

RWC (%) = [(FW-DW) / (TW-DW)] x 100

Where, FW is the sample fresh weight; TW is the sample turgid weight and DW is the sample dry weight.

Physiological data analysis

The data were subjected to analysis of variance and means were compared using least significant difference (LSD).

DNA isolation

Four peanut genotypes were grown in containers in a greenhouse on November 2006. Young healthy leaves were collected at 9 DAE and stored in liquid nitrogen. Leaf tissues were fine-ground with mortar and pestle and the ground tissues were used for DNA extraction by using the

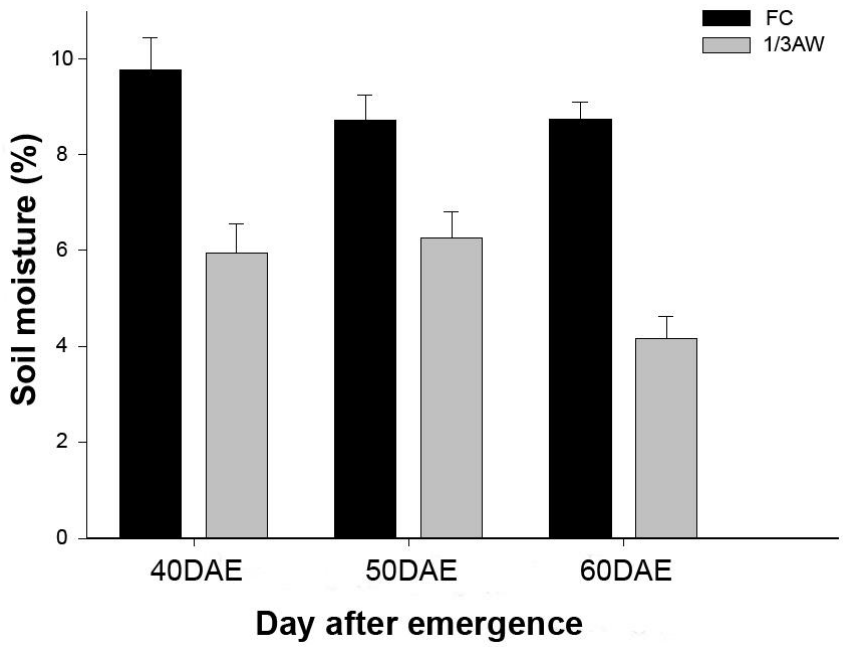

Figure 2. Soil moisture under field capacity (FC) and 1/3 available water (1/3 AW) at 40, 50 and 60 days after emergence (The bar is standard error).

Table 1. SSR primers from different source, number of primers, number of scoring primers and number of polymorphic primers among 4 peanut genotypes.

Source of primer	Primers name	Number of primer	Scoring primer	Polymorphic primer
Cuc et al. (2008)	IPAHM	85	62	31
Moretzsohn et al. (2004, 2005)	Ah , TC, AC	143	93	43
He et al. (2003)	PM	15	12	6
Hopkins et al. (1999)	Ah	12	8	3
Gimenes et al. (2007)	Ah, Ag	10	7	6
Total	-	265	182	89

GenElute Plant Genomic DNA Miniprep kit (SIGMA- ALDRICH, USA). DNA was quantified on a 0.8% agarose gel by visual comparison with lambda DNA standard (Invitrogen, USA) on ethidium bromide stained and subsequently diluted to 5 ng/ µl for PCR.

SSR analysis

665 genomic SSR markers from different sources (Table 1) were used to identify polymorphic among peanut genotypes. PCR reactions for all primers were performed in 5 µl reaction volume in an ABI system 9700 thermal cycler (Applied Biosystem, USA) in 96-well PCR plates (Applied Biosystems, USA), consisting of 2 pmole of each primer, 2 mM $MgCl_2$, 0.1mM dNTPs, 0.1 Unit of Taq DNA polymerase (Qiagen, Germany) and 1X PCR buffer (Qiagen, Germany). Touchdown PCR amplification was employed, and the reaction conditions included 3 min for initial denature cycle, followed by first five cycles of 94°C for 20 s, 60°C for 20 s and 72°C for 30 s with 1°C decrease in annealing temperature per each cycle, then 30 cycles of 94°C for 20 s, constant annealing temperature at 55°C 30 s and 72°C for 30 s followed by a final extension for 20 min at 72°C. The annealing temperatures were applied to all primer pairs.

Electrophoresis and DNA banding analysis

SSR products were separated by electrophoresis on 6% non-denaturing polyacrylamide gels (PAGE) at 650 volt for 2.5 to 3 h in 1X TBE buffer on the Sequi-gen GT sequencing cell electrophoresis (Bio-RAD, USA) with the gel size 38 x 30 cm and 0.4 mm thickness, and then SSR products were visualized through silver staining. The size of fragments was estimated based on 100 bp DNA ladder from Invitrogen (Invitrogen, USA). The presence or absence of amplicons in the genotype examined was scored as 1 or 0, respectively. A quality score for each primer was rated for six levels as recommended by Ferguson et al. (2004). The score ratings were 1 = unambiguous scoring, 2 = allele closed but scoring possible, 3 =

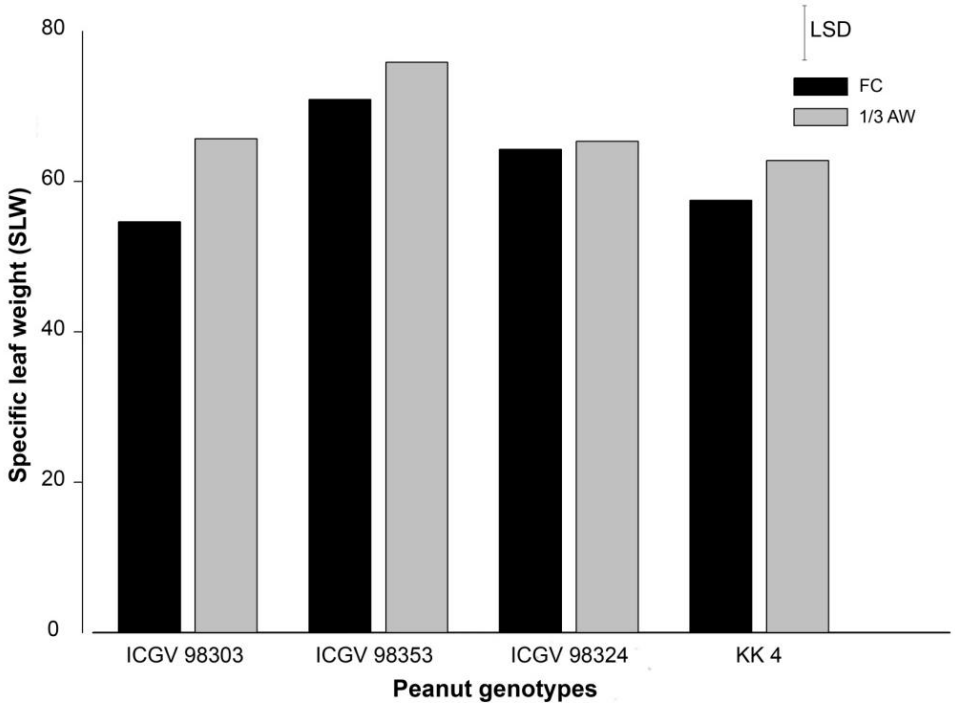

Figure 3. SLW of four peanut genotypes grown under different soil water regime field capacity [FC and 1/3 available water (1/3AW) at 60 DAE]. The vertical bar is LSD = 0.05.

allele too close for accurate scoring when run on PAGE, 4 = weak amplification in at least one locus, 5 = variation due to absent of band and 6 = variation in a fainter, secondary locus but of expected size range.

The polymorphic information content (PIC) of each microsatellite locus was determined as follows;

$$PIC = 1- \Sigma P^2_i$$

Where, P_i is the frequency of the i_{th} allele in the genotypes examined (Weir, 1996). Allelic data obtained in 0 to 1 binary data for all alleles were used for computing the genetic similarity matrix by Dice's coefficient. The UPGMA method was used to construct a dendrogram by using NTSYSpc 2.01 software (Rohlf, 2000). Statistical stability of the branches in the cluster was performed by bootstrap analysis with 1,000 replicates using WINBOOT software (Yap and Nelson, 1996).

RESULTS

Weather data and soil moisture

Weather data were obtained from a meteorological station located near the experimental site. The experiment was conducted during the dry seasons from November 2006 to February 2007. There were maximum rainfalls of 1.0 mm at 17 DAE (Figure 1). The seasonal means of maximum and minimum air temperatures ranged from 30.6 and 18.1°C respectively. Daily pan evaporations ranged from 2.86 to 7.84 mm. Soil moisture contents at 1/3 AW were significantly lower than at FC at 40, 50 and 60 days after emergence (Figure 2).

Physiological analysis

Peanut genotypes were not statistically different for SLW and RWC at 40 and 50 DAE, and the differences between drought-treated plants and normal plants were also not significant for all peanut genotypes (data not present). However, the data indicated that SLW was increased under drought conditions and peanut genotypes were significantly different for SLW under drought and FC conditions at 60 DAE (Figure 3). ICGV 98353 showed the highest SLW, whereas KK 4 had the lowest SLW under drought conditions.

RWC were significantly decreased in all peanut genotypes as affected by drought at 60 DAE (Figure 4). The differences among peanut genotypes were also significant under drought conditions, whereas all peanut genotypes performed rather similar under field capacity conditions. ICGV 98324 had the highest RWC and KK 4 had the lowest RWC.

SSR analysis

265 SSR markers were screened and 182 genomic SSR markers were clearly visualized on PAGE and could be score (quality score as 1 and 2). 23 markers gave the bands of alleles that were close together and scoring of the band was very difficult. 29 markers gave the bands that were poorly visualized, and 31 markers had variation due to absent of band (Figure 5). The markers that gave

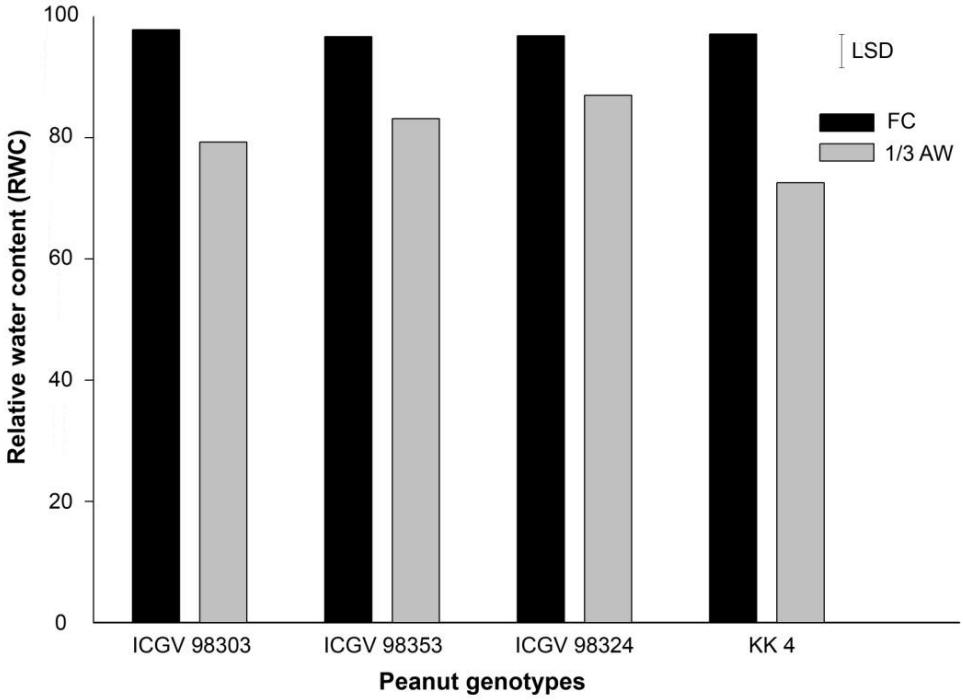

Figure 4. RWC of 4 peanut genotypes grown under different soil water regime [FC and 1/3 available water (1/3AW) at 60 DAE). The vertical bar is LSD = 0.05.

the clear bands (quality score as 1 and 2) were used, and the others were excluded from the analysis.

Out of 182 clearly-visualized markers, 89 markers could detect polymorphism among peanut genotypes (48.9%). The numbers of alleles per locus of polymorphic markers ranged from 1 to 6 with a mean of 2.7 alleles. The polymorphic markers consisted perfect, imperfect and compound repeats. Dinucleotide repeats were most frequently observed (Table 2). The PIC values ranged from 0.38 to 0.75 with an average of 0.48 (Table 2). Out of the 89 polymorphic markers, there were 20 makers having specific bands for KK4 genotype (Table 3). The data from 89 SSR polymorphic markers then were scored as present (1) or absent (0) binary data. The genetics relationships among peanut genotypes were estimated using Dice's coefficient. The bootstrap was performed with 1,000 replicates to support the dendrogram, and the values ranged from 61.3 to 98.9% (Figure 6). The dendrogram showed that KK 4 (susceptible genotype) was an isolated cluster distinct from other genotypes, whereas ICGV 98324 and ICGV 98303 were assigned into the same cluster furthest from the KK 4.

DISCUSSION

Weather conditions in this study provided normal growth of peanut. The most appropriate evaluation time of SLA would be at 60 DAE. Evaluation earlier than 60 DAE would results in low variation for this trait. This may be due to drought tolerance nature of peanut that takes longer time for responses to drought (Holbrook and Stalker, 2003). Songsri et al. (2008) also found that the SLA under 1/3 AW and 2/3 AW were not significantly different at 37 DAE because of early sampling date. At 60 DAE, peanut genotypes were statistically different for SLW and RWC. The results indicate that these peanut genotypes were different in drought resistance.

As SLW is related to leaf thickness, peanut genotypes with high SLW could maintain higher photosynthetic capacity. Peanut genotypes with high SLW also had higher chlorophyll contents and Rubisco enzyme. These enzymes are well known to involve in photosynthesis pathway (Arunyanark et al., 2009; Nautiyal et al., 2002).

Craufurd et al. (1999) suggested that peanut genotype with low SLA could maintain higher water use efficiency (WUE) under water limited conditions. Under drought conditions, SLA has negative correlation with WUE and harvest index. SLA also has high heritability and less effect of genotype x environment interaction (Songsri et al., 2008), and, thus, it is possible to use SLA to evaluate drought resistance in peanut. In previous study, Reddy et al. (2003) found that RWC values ranging from 85 to 90% under well-irrigated conditions were dropped to 30% under drought. Nautiyal et al. (1995) suggested that drought tolerance in peanut could be characterized by the maintenance of RWC under drought conditions. ICGV 98324 could maintain the highest RWC and it should

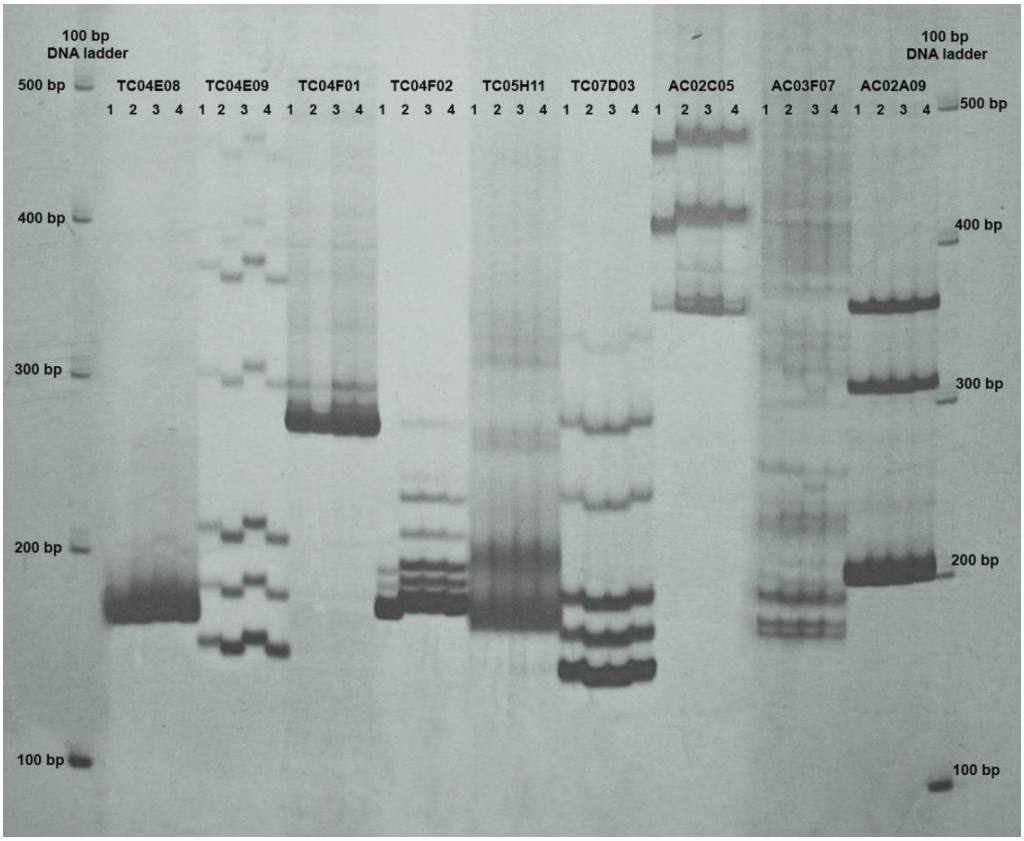

Figure 5. DNA profile obtained from SSR markers of KK 4 (1), ICGV 98324(2), ICGV 98303 (3) and ICGV 98353 (4) on 6% non-denature polyacrylamide gel stained with silver and determine the allele sizes base on 100 bp DNA ladder (L).

Table 2. Number of alleles, polymorphic information content value (PIC) and SSR-motif of the polymorphic SSR primers among the four peanut genotypes.

Primer name	Number of allele	PIC	Motif
Ah7	2	0.50	na
Ah11	4	0.63	na
Ah19	5	0.75	na
Ah30	2	0.38	na
Ah-075	2	0.50	$(ACA)_6$
Ah-097	2	0.38	$(AAC)_5$
Ah-193	4	0.75	$(AAC)_5(GA)_{24}$
Ah-229	3	0.63	$(ACT)_6(TCT)_9$
Ah282	2	0.38	na
Ah-590	3	0.63	$(TTC)_8(TTC)_4(CTT)_{10}(TTC)_4(TCT)_4(CTT)_5(TTC)_4(GAA)_4$
Ah-594	3	0.63	$(AAC)_{10}$
Ah-692	3	0.63	$(CAA)_6$
Ah-745	3	0.38	$(TTG)_4(TTG)_5(AGA)_4$
Ah4-24	2	0.38	$(ATA)_{17}$
Ah4-02	2	0.38	$(GA)_{19}$
Ah4-11	2	0.50	na
Seq15D06	2	0.50	$(CTT)_5(CT)_8$

Table 2. Contd

Seq16C07	3	0.63	$(CT)5(TC)_{18}$
Seq04E04	2	0.38	$(AG)_{16}$
Seq04B11	3	0.63	(CA)7
TC01E01	6	0.75	$(GA)_{29}$
TC03G01	3	0.38	$(TC)_{14}(TC)_{12}$
TC03G05	2	0.38	$(GAG)_4(CTT)_{15}(TC)_{23}$
Ag140	2	0.50	na
TC11A02	2	0.38	$(AG)_{19}$
TC07G10	2	0.38	$(GA)_{17}$
TC09C06	3	0.38	$(AG)_{18}(AG)_{49}$
TC09C08	2	0.38	$(GA)_{22}$
TC09B08	3	0.63	$(GA)_{22}$
TC11E04	2	0.38	$(AG)_{23}$
AC1C11	2	0.38	$(AGA)_4(GT)_{16}$
AC2C12	2	0.38	$(TA)_6(TG)_{20}(GT)_6$
AC1D11	2	0.50	(TG)20
AC1G11	6	0.63	$(TA)_5(TG)_{27}$
AC2C08	4	0.63	$(AT)_6(TG)_{31}$
AC02B03	4	0.63	$(CT)_9(CA)_{21}$
XIPAHM552	2	0.38	na
XIPAHM354	2	0.38	(GA)18
XIPAHM395	2	0.38	$(GA)_{14}$
XIPAHM407c	2	0.38	$(GA)_{17}$
XIPAHM455	2	0.38	$(TA)_5(TG)_{16}$
XIPAHM468	3	0.38	$(GA)_{15}$
XIPAHM475	3	0.38	$(GT)_7(GA)_{12}$
XIPAHM509	2	0.38	$(CA)_{25}$
XIPAHM524	3	0.38	$(GA)_{20}$
XIPAHM531	2	0.50	$(TAC)_7$
XIPAHM659	3	0.38	$(GA)_{18}$
XIPAHM684	2	0.38	$(TG)_{10}$
XIPAHM023	2	0.50	$(CA)_{17}(TA)_3$
XIPAHM82	2	0.38	$(GA)_{15}$
XIPAHM93	3	0.63	$(CT)_{15}$
XIPAHM108	3	0.63	$(TC)_{18}$
XIPAHM123	2	0.38	$(GA)_{18}$
XIPAHM229	2	0.38	$(CA)_{14}(CA)_3$
XIPAHM176	2	0.38	$(G)_5(GA)_{18}$
XIPAHM254	2	0.38	$(GA)_5(GA)_{20}(GA)_4$
XIPAHM255	4	0.75	$(AGGG)_3(AG)_{23}$
XIPAHM272	2	0.50	$(TA)_7(GT)_{12}(TG)_7$
XIPAHM287	2	0.38	$(TG)_{16}(AG)_{22}$
XIPAHM290	2	0.38	$(TA)_3(CA)_3(CA)_5(TA)_8$
XIPAHM302	2	0.38	$(AG)_{14}(AG)_8$
XIPAHM320	4	0.63	$(GA)_{11}$
XIPAHM333	2	0.50	$(TG)_{15}$
XIPAHM165	3	0.38	$(GA)_{13}$
XIPAHM171c	4	0.63	$(GA)_{16}$
XIPAHM219	2	0.38	$(TG)_{15}$
XIPAHM245	3	0.63	$(GT)_{13}$

Table 2. Contd

PM32	3	0.38	$(CT)_{15}$
PM35	2	0.38	$(GA)_{18}(GAA)_2$
PM36	4	0.63	$(GA)_{18}$
PM42	2	0.38	$(GA)_3GAAA(GA)_{14}$
PM45	2	0.50	$(GA)_{16}$
PM69	2	0.38	na
TC00A01	4	0.75	$(AG)_{29}$
TC01A08	6	0.75	$(AG)_{30}$
TC01D12	3	0.38	$(TC)_9$
TC01E05	2	0.50	$(TA)_6(TG)_{20}(GT)_6$
TC02B09	2	0.38	$(TC)_{27}$
TC02D08	3	0.50	$(CT)_{25}$
TC02G05	2	0.38	$(GA)_{35}$
TC03B04	3	0.38	$(TC)_{23}$
TC04C11	4	0.63	$(CT)_{18}(TC)_{10}$
TC04E09	2	0.50	$(TC)_{22}$
TC04F02	2	0.38	na
TC04F10	3	0.38	$(TTC)_{33}$
TC04G05	5	0.75	$(CT)_{33}$
TC07D03	2	0.50	$(GA)_{16}$
AC2C05	3	0.38	$(TG)_{17}$
TC01B02	2	0.38	$(CT)_{25}$

na = Not available.

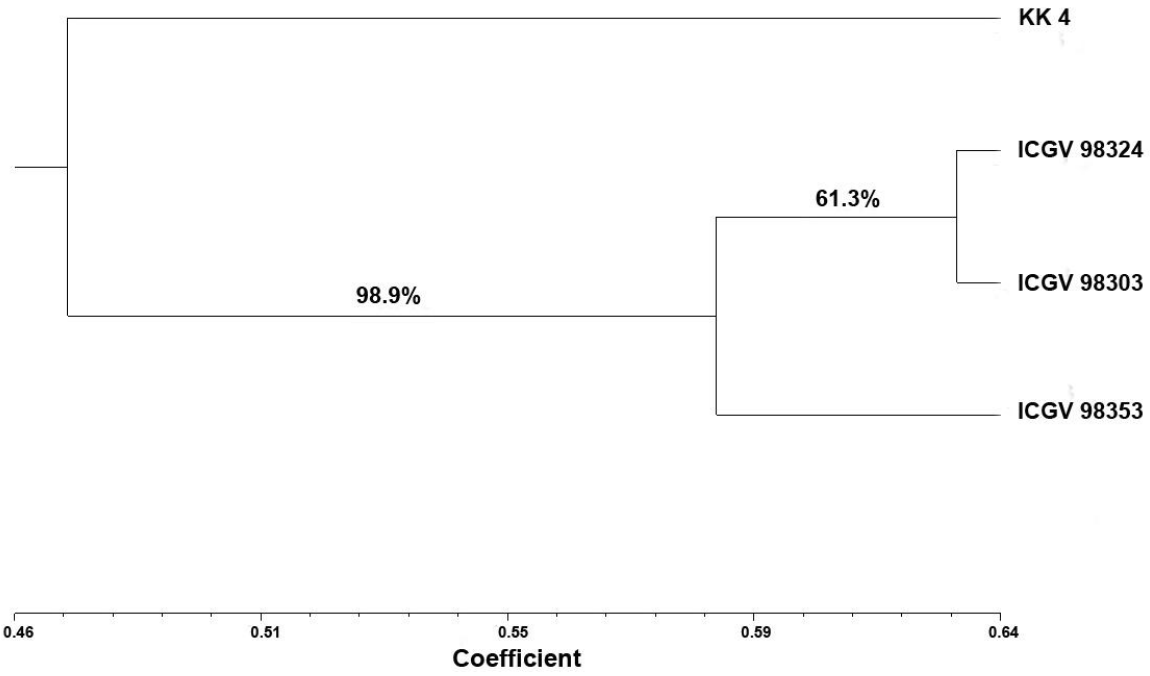

Figure 6. SSR based phylogenetic tree of the four peanut genotypes. The number on the branches represent the percentage of 1,000 bootstraping.

Table 3. Polymorphic SSR primers specific to KK4.

Primers name	PCR sized (KK4)	Forward	Reverse
Ah-745	210	TGTTGTTCTGCTCCTGCTTTTG	ATTCGGACCAAAATGTCCCTTC
Seq04B11	220	CATGCCGAAATGGAATGAA	AAGTGGGCGAGTGAGAACAG
TC03G05	145, 220	GATCCCAAGTCTCCAGAGGA	AACAACAAGGAGGCAGAGGA
AC1C11	196	CTCCCACACCAAACTTAAAAGC	CCCACTCCTATAAATACCCCTCTT
AC2C12	220	TATCGAGCCGAATATGAAT	GCAGGATTTTGTAATTGAGAG
XIPAHM82	300	CCATATCATAGCCGCCAAGT	TACATCCACGATGCAGAAGG
XIPAHM123	142	CGGAGACAGAACACAAACCA	TACCCTGAGCCTCTCTCTCG
XIPAHM219	116	TCTCTTTTGTGTATTTTGGGCTA	AGCCTGCGAAACTAAGGTTG
XIPAHM229	148	TCAGCCTGCGAAACTAAGGT	TGGAGAACTAGGATCTCTTTTGTG
XIPAHM287	202	TCTAACCCTTCGGTTCATGG	TCACTATCCCATCCCTGCTC
XIPAHM290	298	CCACCGCTGATGTGTAATTGTA	GACGTGTAGTTGAAAACAACAGTATCA
XIPAHM320	125,275	ACTTCGCGGGTGATAGAGTG	CGTCCCAATATCCCTTCAGA
XIPAHM395	210	CAGAGTCAATGGCAGCGTAG	TCCTTCCCTCATCTAAAACCAA
XIPAHM455	178	TGCAGAGACTTGTATTTTGAGG	AAGCCTTTGCGAATATAACC
XIPAHM475	300	GTGATTTCCTGGTTGGTGCT	AGCCTCAGCTGGTTTTGCT
PM32	145,250	AGTGTTGGGTGTGAAAGTGG	GGGACTCGGAACAGTGTTTATC
TC01D12	210,245	CCCTTTCATTCTCCCTTTCC	TTCTCCTGCACTAGGTTTCCA
TC03B04	230	GAAGAAGAAGTCACTGCGGC	AAGCTAGTTTCTGATTAAAGCACCA
TC04F02	175	GCACTGCACCCCAATCTCTA	GATGGGTGGTTTGGTGTCTC
AC2C05	415,480	CAAGGAAGCGTGAATTGTTAG	TGTGGACTATGCTTGTCATGTT

have normal growth under drought stress. KK 4 showed the lowest SLW and RWC; whereas ICGV 98353 showed the highest SLW and ICGV 98324 had the highest rela-tive water content. The results indicate that physiological characters could differentiate peanut genotypes into two groups. ICGV 98353 and ICGV 98324 are known for drought resistance and KK 4 is known as drought sus-ceptible. Therefore, the question underlying the research project is that these peanut genotypes previously showing the difference in RWC and SLW are also different for molecular markers. In this study, 20 markers were speci-fic to KK 4, confirming the difference between drought sensitive and drought tolerant genotypes. Direct compa-rison of different studies is not possible because, to the best of our knowledge, there are no previous report on the use of molecular markers to differentiate peanut genotypes with difference in SLW and RWC. However, in this study, the use of SSR markers to differentiate peanut genotypes with difference in SLW and RWC was successful.

Indirect comparison of the results of different studies for different traits would be possible. Compared to previous report, Mace et al. (2006) also reported high level of poly-morphism between cultivated peanut for this type of markers, in which 12 markers from 23 SSRs (52%) could detect polymorphism among cultivated peanut germ-plasm resistant to rust and late leaf spot diseases.

The bootstrap in this study was rather low possibly due to the narrow genetics based in peanut. Higher bootstrap would be obtained by adding more polymorphic SSR markers (Barkley et al., 2007).

Conclusion

Drought could increase SLW and decrease RWC. ICGV 98353 had the highest SLW under well water

and water limited conditions. ICGV 98324 could maintain the highest RWC under drought conditions. KK4 had the lowest SLW and RWC under water limited conditions. The use of SSR markers to differentiate peanut genotypes with difference in SLW and RWC was successful. ICGV 98324 and ICGV 98303 were grouped in the same cluster furthest from the KK 4. Based on physiological traits and markers data, KK 4 seems to be most sensitive to drought compared to the previously known drought resistant genotypes. The data from the present study could be useful as a source of variation forconstructing the mapping population different in both phenotypic and genotypic value for QTL analysis of physiological trait related to drought stress in breeding program.

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