

## RELATIVE WATER CONTENT AS AN INDEX OF PERMANENT WILTING IN GROUNDNUT UNDER PROGRESSIVE WATER DEFICIT STRESS

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**Abstract:** *The relationship between relative water content (RWC) and permanent wilting was studied in 21 Spanish groundnut cultivars by imposing 60 days of progressive stress (DPS) by withholding irrigation 24 days after emergence (DAE) and periodical recording of soil moisture content, soil temperature, leaf RWC and wilting symptoms and then re-watering and recording of rejuvenation during Summer season. With increasing water deficit stress, the leaf RWC declined progressively in all cultivars with mean values of 92, 85, 77, 69 and 61 at 10, 25, 35, 50 and 60 DPS, respectively. Amazingly, even after 60 DPS, the RWC was above 60 in 13 out of 21 cultivars of which 11 cultivars showed <25% visual wilting ( $VW_p$ ) and of these seven cultivars i.e. TPG 41, ICGV 86590, TG 37A, Girnar 3, AK 159, GG 4 and DRG 12 showing <10% permanent wilting ( $PW_p$ ) are promising. The study conclude that lower limit of RWC in groundnut leaves causing permanent wilting though varied with cultivars, the groundnut plants could survive moisture deficit up to 60% RWC and further reduction enhanced permanent wilting. A strong inverse relation between RWC at 60 DPS and  $VW_p$  ( $r = -0.74^{**}$ ) and RWC at 60 DPS and  $PW_p$  ( $r = -0.76^{**}$ ) respectively, indicate that RWC at the 60 DPS determines the mortality of groundnut plants undergoing severe moisture deficit stress.*

**Key words :** Groundnut, Water deficit stress

### INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important food legume and oilseed crop of the world grown on about 24 million ha of land in about 120 countries mostly in tropics and subtropics of arid and semi-arid regions where the availability of water is a major constraint on yield and crop frequently suffers drought of various spells and intensities [1]. As a result, the groundnut productivity is less than 1000 kg ha<sup>-1</sup> in more than 50% of groundnut growing countries. Final yield suffers severely because of reduced plant strength due to permanent wilting under severe water deficit stress. Plants adopt various defence mechanisms in response

to drought, which are regulated through internal plant water status and even short-duration fluctuations in plant water status during the reproductive period could adversely affect the development and function of reproductive organs [2]. The effectiveness of a particular physiological trait as selection criteria in a breeding programme depend on the rapid assessment of the plant at a critical stage, using small quantities of plant material. Development of stress-tolerant cultivars is a time consuming and tedious process with partial success because the acquisition of drought tolerance in plants is a complex phenomenon including physiological, molecular and biochemical modifications. Therefore, identification of simple indicator is required, which can

be easily employed in the plant breeding programmes. Relative water content (RWC) is beyond question the most appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit [3]. Crop response to water deficit often include physiological changes that minimize water loss, such as closing stomata and reducing leaf surface area by leaf rolling. An additional response that has been given less attention is canopy wilting [4]. Peanut genotypes differ in canopy wilting under different regimes of available soils water [5]. The concept of permanent wilting point (PWP) introduced in the early 1910s is a constant (characteristic) of the soils and independent of environmental conditions. It is the largest water content of soils at which indicator plants, growing in those soils, wilt and fails to recover when placed in a humid chamber [6]. But as, PWP is a soil physical property and there are many limitations in its measurement at field condition. Hence, there is a need to develop relation between the RWC and permanent wilting in groundnut. Thus, a study was aimed at seeking information on leaf RWC and its impacts on visual wilting symptoms and permanent wilting under deficit soils water status.

## MATERIALS AND METHODS

A field experiment was conducted during summer 2012 (January- June) using 21 Spanish groundnut cultivars at the research farm of Directorate of Groundnut Research (DGR), Junagadh, Gujarat (lat 21° 31' N, Long 70° 36' E) India in Vertic Ustochrept soils (Table 1). The experiment was laid in a completely randomized block design with three replications. The 21 groundnut cultivars were sown in 3 plots of 4x3 m with nine rows /plot (seven effective rows + two borders) at 45 cm row to row and 10 cm plant to plant spacing and irrigated

till 90 DAE. The water deficit treatment was withdrawn by applying irrigation on the completion of 60 DPS by applying two irrigations, first on 61 and a second on 65 DPS. The soils moisture content was measured using gravimetric method at two different depth viz. 0-15 cm and 15-30 cm depth. Soils temperature at 5 and 15 cm depths was measured at 0930 and 1600 hr throughout crop growth period. To avoid day to day variation, the mean temperature of the entire standard week in which the observation of RWC was recorded was considered to be the soils temperature of the day of observation.

The leaf relative water content was estimated as  $RWC (\%) = ((FW - DW) / (TW - DW)) * 100$ . Where, FW fresh weight, DW dry weight and TW is turgid weight.

The RWC were recorded at 10, 25, 35, 50 and 60 DPS in three replications. A leaf sample was made up of three individual third leaf from main axis collected from different plants. For analysis, the mean of three leaves was treated one replication. Canopy wilting on the verge of visible wilting symptoms was calculated on per cent basis through a modification in wilting scoring method of for soybean given by King et al. [7]. A score in a range of 1 to 5 was assigned to characterize the visual symptoms of wilting due to water deficit stress in morning hours at 60 DPS. A score of 1 indicated no wilting symptom, 2 for initiation of wilting; 3 for most of the leaves wilted but not dried, 4 was assigned to a visibly completely dried plant and a score of 5 to dead plants. Plants with score more than 3 were considered as visually wilted and the per cent visual wilting ( $VW_p$ ) was calculated as:  $W_p = (\text{number of visually wilted plants} / \text{total number of plants in a population}) * 100$ . The permanent wilting was calculated as per cent of total population which did not recover after 7 days after stress withdrawal as per the following formula:

$$PW_p (\%) = \frac{(\text{number of permanently wilted plants after stress withdrawal})}{(\text{total number of plants in population before stress withdrawal})} \times 100$$

The rejuvenation capacity defined as difference between  $VW_p$  and  $PW_p$  in per cent was calculated as  $RC (\%) = ((VW_p - PW_p) / (VW_p)) * 100$

at 0 and five days after sowing. Later on two more irrigations were applied; the last on 24 DAE and then the irrigation was completely with-hold for 66 days so that the actual progressive water deficit stress period started from 30 DAE (flowering stage) and continued

Statistical analysis of the results were analysed by randomised block design with three replications and the critical differences were calculated to assess the significance of treatment means where the “F” test was found significant at 5%.

## RESULTS AND DISCUSSION

**Soil moisture and temperature:** The soil moisture content progressively decreased from 16.8 at 10 DPS to 2.3% at 60 DPS in 15-30 cm soil layer and from 15.3 at 10 DPS to 0.39% at 60 DPS in 0-15 cm soil layer with progressive increase in stress (Fig. 1a).

The mean day time temperature at 5 cm depth which was 33.8 °C at 10 DPS increased to 38.0 °C at 60 DPS whereas a reverse trend was found at 15 cm depth where soil temperature 40.3°C at 10 DPS decreased to 38.3 °C at 60 DPS (Fig. 1b).

**Leaf relative water content:** The RWC of leaf in groundnut cultivars declined with the progression of water deficit stress with mean value of 92, 85, 77, 69 and 61 on 10, 25, 35, 50 and 60 DPS, respectively (Fig. 2). There was a significant difference in leaf RWC in groundnut cultivars. At 10 DPS the RWC was more than 90 in all cultivars except TG 37 A (89) and AK 159 (87). The mean RWC decreased at 25 DPS was highest in VRI 3 (87) followed by GG 4 (87) and lowest in Chico (81). On 35 DPS, the RWC was highest in JAL 42 (80) and lowest in VG 9521(74). The RWC remained above 60 in all cultivars at 50 DPS, and was again highest in JAL 42 (71.9) followed by ICGV 86590 (70.5) and lowest in GG 5 (65.5). The mean RWC at 60DPS remained above 60 in 60% of cultivars with more than 60 in JAL 42 (63.4), DRG 12 (62.9), Girnar 3 (62.6), GG 4 (69.2) and TPG 41 (62.8), whereas with the lowest value in VG 9521 (57.3) followed by GG 5 (57.5) and JGN 23 (58.4). Decrease in RWC under soil moisture deficit condition in groundnut is reported by Nautiyal et al. [8]. The decrease in RWC is associated with decreased water uptake under deficit soil moisture condition.

### Visual wilting symptoms and permanent wilting:

A significant difference in % visual wilting ( $VW_p$  %) at 60 DPS was observed (Fig. 3) with the highest in GG 2 (42%) and the lowest in ICGV 86590 (5%). The  $PW_p$  ranged from 4.1% in TPG 41 to 25.7% in GG 2 with the mean value of 12.6% of visibly wilted plants which did not recover after 60 days of progressive water deficit stress followed by seven days of rehydration. Interestingly, cultivars VG 9521, SB IX, Chico and GG 5 showed >30%  $VW_p$  and > 20%  $PW_p$ . On the other hand TPG 41, ICGV 86590, TG 37A, Girnar 3, AK 159, GG 4 and DRG 12 showed <15%  $VW_p$  and <10%  $PW_p$ .

The groundnut showed visual wilting when grown at 2/

**Table 1:** Physicochemical properties of the experimental soils

Soils Characteristics	Soil depth	
Vertic-stochrept Calcereous Medium Black Clayey Soils	0-15 cm	15-30 cm
Mineralogical		
Sand	22.40	20.30
Silt	14.00	15.77
Clay	63.60	64.20
Physical		
Field Capacity	30.35	30.25
Permanent Wilting Point	14.4	13.95
Bulk Density	1.44	1.46
pH	8.5	8.5
Electrical Conductivity	0.16	0.14

**Table 2:** Correlation between RWC at 60 days of progressive stress, visual wilting ( $VW_p$ ) and permanent wilting of plants ( $PW_p$ ) in groundnut under progressive water deficit stress (DPS)

	RWC 60 DPS	$VW_p$
$VW_p$	-0.74**	
$PW_p$	-0.76**	0.90**

3 available water and more severe wilting symptoms at 1/3 available water in the afternoon [5]. The mechanisms that contribute to differential canopy wilting is important for determining if delayed wilting could increase yields under drought and for determining under what conditions yields might be affected. The varietal differences for canopy wilting is reported to be associated with rooting traits, such as rooting depth, lateral rooting, or root-length density, that influence the ability to extract water from the soil profile [9].

There was a negative correlation between RWC and  $VW_p$ (-0.53) and RWC and  $PW_p$ (-0.54) and the relationship was even more stronger between RWC at 60 DPS and  $VW_p$  (-0.74) and RWC at 60 DPS and  $PW_p$ (-0.76) (Table 2).

At 60 DPS the RWC was more than 60% in 13 out of 21 groundnut cultivars while at the same time the soil moisture content was 0.4% and 2.3% at 0-15 and 15-30 cm depth respectively with a mean day time soil temperature of 38.0°C at 15 cm and 38.3°C at 5 cm depth which indicated that the plants were able to absorb required moisture from the deeper soil layers through deep root system. Soil water contents could affect root depth and distribution in groundnut and varietal difference for root growth and the changes in root distribution pattern in response to drought by growing roots deeper into the soil is well documented [5,10]. Groundnut roots effectively extracted soil water to a depth of at least 180 cm in fine sand soil as roots in

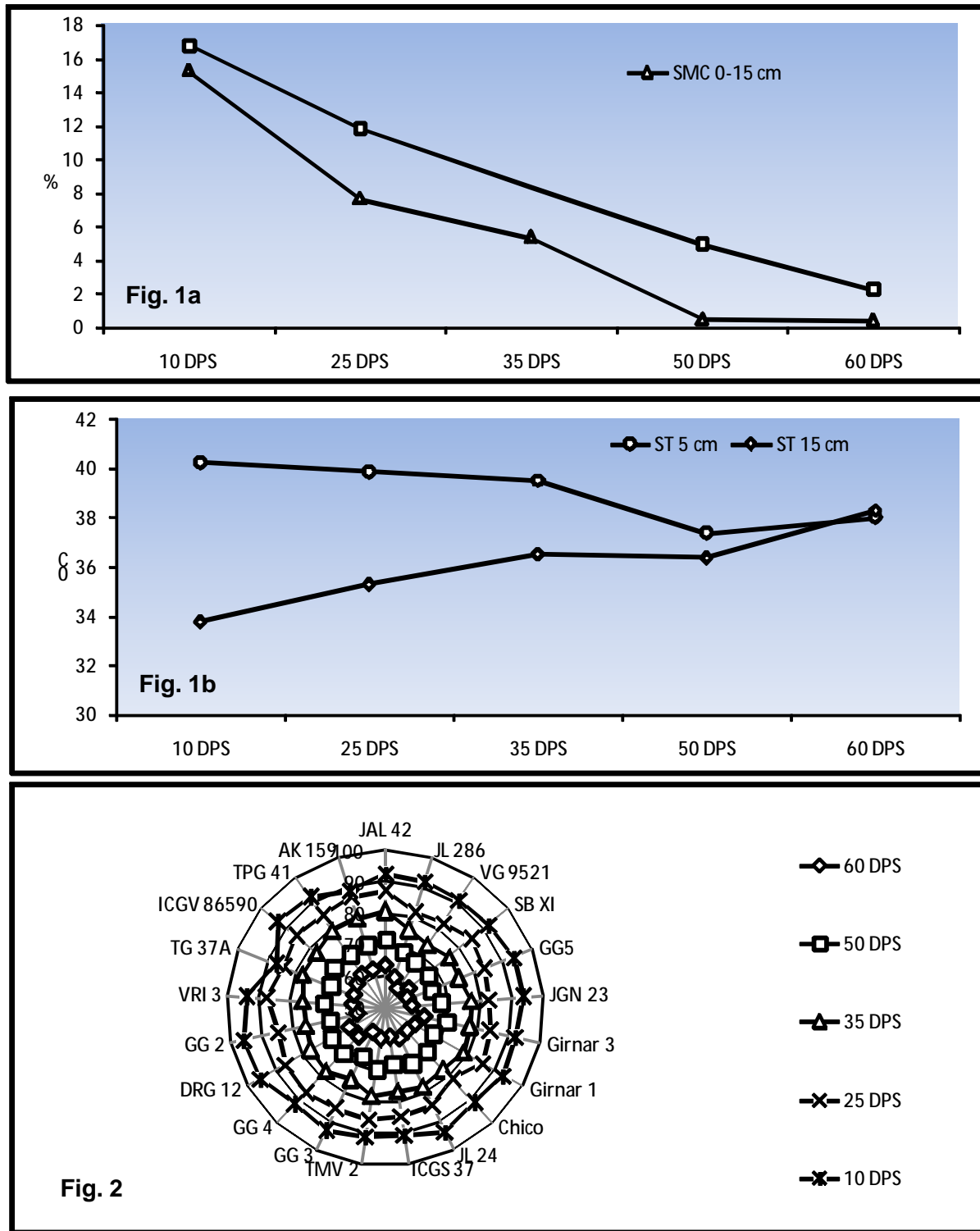


Fig. 1. (a) Soil moisture content of the experimental field under progressive water deficit stress (DPS).

Fig. 1. (b) Soil temperature of the experimental field under progressive water deficit stress (DPS).

Fig. 2. Leaf relative water content (%) under progressive water deficit stress in groundnut.

lower depths continue to grow deeper even though vegetative growth appears to stop [11]. Through roots, plants affect soil moisture regime. The more prominent rooting system, gives rise to efficient utilization of water and nutrients to the plant. The penetration of root creates

new channels within soil and makes the soil of adjacent area compact and rigid which results information of soil structure and loosening of soil and increases the rate of infiltration [12]. In calcareous soils of Junagadh the root growth goes more than 2.0 meter under water

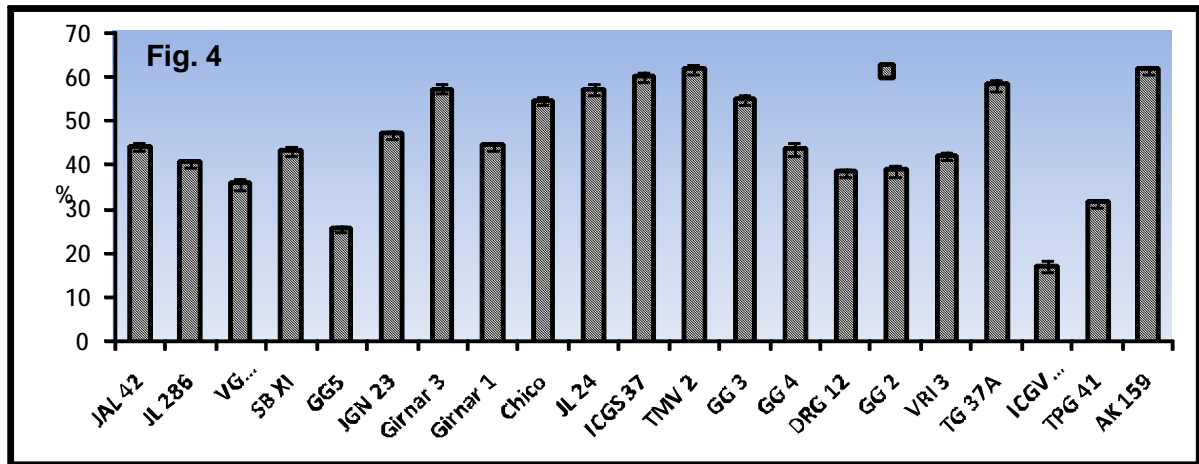
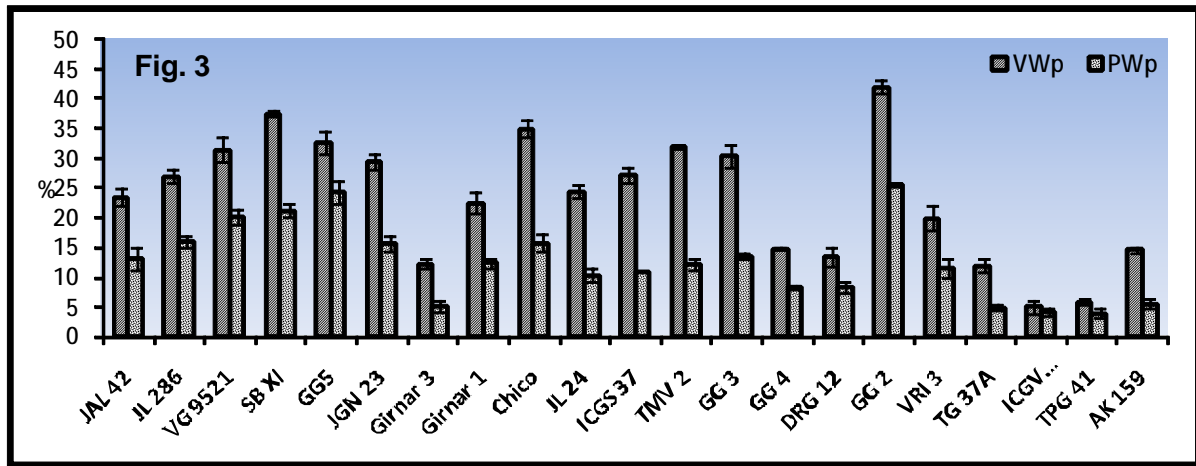


Fig. 3. Visual wilting ( $VW_p$ ) and permanent wilting ( $PW_p$ ) of plants in groundnut at 60 days of progressive stress (DPS).

Fig. 4. Rejuvenation capacity (%) in groundnut after 60 days progressive water deficit stress.

stress condition (data unpublished). The rejuvenation capacity, defined as difference between  $VW_p$  and  $PW_p$  as per cent of the total  $VW_p$  remained high in those cultivars which were having RWC more than 60 % at 60 DPS (Fig. 4).

The  $PW_p$  was 53 % of the  $VW_p$  which indicates that, even if the groundnut plant seems to be wilted, about 50 % of visually wilted plants can rejuvenate if the moisture is supplied even after a 60 days long dry period. The higher rejuvenating capacity found in some of the groundnut cultivars is because of higher wilting as an adaptive mechanism which resulted in more  $VW_p$ . In another ways, groundnut plants can withstand moisture deficit condition to the lowest limit 60% RWC below which the chances for visibly wilted plant to become permanently wilted may increase by more than 60%. However, the lowest limit of RWC in leaves, which can create a desiccation effect, at which none of the visibly wilted plant can rejuvenate after stress withdrawal vary with cultivars.

n very dry soils, the water potential ( $\Psi_w$ ) may fall below the permanent wilting point. At this point the water potential of the soil is so low that plants cannot regain turgor pressure even if all water loss through transpiration ceases. This means that water potential of the soils ( $\Psi_w$ ) is less than or equal to the osmotic potential ( $\Psi_s$ ) of the plant. Because cell  $\Phi_s$  varies with plant species, the permanent wilting point is clearly not a unique property of the soils; it depends on the plant species as well. When a soil dries, its resistance to the flow of water increases sharply, particularly near the permanent wilting point (usually about  $-1.5$  MPa) and plants cannot regain turgor pressure even if all transpiration stops. Because of the very large soil resistance to water flow, water delivery to the roots at the permanent wilting point is too slow to allow the overnight rehydration of plants that have wilted during the day. Rehydration is further hindered by the resistance within the plant, which has been found to be larger than the resistance within the soil over a wide range of water

deficits [13]. Several factors may contribute to the increased plant resistance to water flow during drying. As plant cells lose water, they shrink and when roots shrink, the root surface moves away from the soil particles that hold the water, and the delicate root hairs may be damaged. Water potential as an estimate of the energy status of plant water is useful in dealing with water transport in the soil-plant-atmosphere continuum. However, it does not account for osmotic adjustment (OA) which is a powerful mechanism of conserving cellular hydration under drought stress and RWC expresses also the effect of OA in this respect. For the same leaf water potential two different cultivars can have different leaf RWC, indicating a corresponding difference in leaf hydration, leaf water deficit and physiological water status. Hence RWC is an appropriate estimate of plant water status in terms of cellular hydration under the possible effect of both leaf water potential and OA. Leaf water content representing plant water status gives a biological baseline or reference and it is recognized as the major determinant of metabolic activity and tissue or organ survival.

From the study, it is concluded that mere drying of the upper soil layers (up to 30 cm) below theoretical PWP does not warrant permanent wilting of all groundnut plants in calcareous medium black clayey soils in field condition as its roots go beyond that depth. The lower limit of RWC in groundnut leaves causing permanent wilting though varied with cultivars, it could survive moisture deficit up to 60% RWC and further reduction enhanced permanent wilting. Groundnut cultivars TPG 41, ICGV 86590, TG 37 A, Girnar 3, AK 159, GG 4 and DRG 12 having >60% RWC, <15 %  $VW_p$  at 60 DPS and <10%  $PW_p$  which could survive drought period of 60 days (30-90 DAE) can become the hope of the farmer. However, the groundnut cultivars VG 9521, SB IX, GG 5 and GG 2 having <60% RWC and >30%  $VW_p$  at 60 DPS which also showed >20%  $PW_p$  were most sensitive.

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