Indian Journal of Agricultural Sciences **80** (12): 1095–7, December 2010

Leaf water content—a simple indicator of drought tolerance in crop plants*

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Received: 26 June 2009; Received accepted: 18 September 2010

Key words: Drought tolerance, Leaf water content

The traditional approach to plant breeding, relying heavily on generating and widely evaluating large numbers of crosses and progeny lines, and guided by quantitative genetic models will undoubtedly continue to be used with success in favourable environments. However, objective assessments of the effectiveness and efficiency of these breeding procedures in stressful environments are difficult. Physiological research can impact on effectiveness and outcomes of plant breeding in stressful environments, but it needs to be integrated with traditional approach to be adopted and to be of value. But most physiological processes used as screening tests in breeding programmes are too slow and complex to be suitable for testing large numbers of plant genotypes. The utility 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. Identification and development of stress-tolerant cultivars is a time consuming and tedious process with limited 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. Plants adopt various defence mechanisms in response to drought or water-stress, which are regulated through internal plant water status. Even short-duration diurnal fluctuations in plant water status during the reproductive period could adversely affect the development and function of reproductive organs (Kumar *et al*. 2006, Omae *et al.* 2005a). 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. Plant water status during midday hours is very critical for plant performance (Kumar *et al*. 2005, 2008, Omae *et al*. 2005b, 2007). The study was, therefore, aimed at seeking information on genotypic diversity for leaf water content during midday hours (1200–1400 hr) and its association with complex physiological traits and seed yield, so that it can be used for screening large populations to accelerate the breeding process.

Experiments were conducted under controlled and field conditions at CCS Haryana Agricultural University, Hisar, India and Tropical Agriculture Research Front (JIRCAS), Ishigaki, Okinawa, Japan. At Hisar (India), 25 genotypes of cowpea (*Vigna unguiculata* L. Walp.) were grown in drought plots (30 m× 6 m× 2 m) under rainfed conditions in randomized block design and 6 greengram (*Vigna radiata* L. R. Wilczek) genotypes in micro-plots (6 m × 1 m × 2 m) with 2 irrigation levels (watered regularly and drought imposed after seedling establishment) in split-plot design each crop with 3 replications in the summer season of 2006 and 2007. Wheat (*Triticum aestivum* L. emend Fiori & Paol.) was grown under field conditions in a split-plot design with 2 dates of seeding (21 November and 22 December 2006), 3 irrigation levels (watered and drought imposed at ear emergence and anthesis) in the main plots and 4 genotypes in the sub-plots with 3 replications. Snap bean (*Phaskolus vulgaris* L.) was planted in a net house at Ishigaki (Japan) in randomized block design with 16 treatment combinations comprising 8 genotypes and 2 irrigation levels (irrigated and no post-sowing irrigation) with 3 replications in 2004 and 2005. All physiological parameters were measured between 1200 and 1400 hr (ie midday). Leaf water content usually expressed and termed as leaf relative water content (RWC) was estimated by the following equation:

$$RWC (\%) = (FW–DW)/(SW–DW) \times 100$$

where (FW) fresh, oven-dry (DW) and water-saturated (SW) weight of the leaf discs. A sharp cork borer was used to take 8 leaf discs 7 mm in diameter by avoiding the midrib and major veins in the leaf. Water saturation was determined after the leaf discs were floated on distilled water for more than 4 hr in darkness. Samples were then dried in an oven at 65°C for 8 hr to record oven dry weight. Rate of photosynthesis (P_N) and intercellular CO_2 concentration (C_i) was determined by a potable photosynthesis system (LI 6400, LI-COR, Lincoln, Nebraska, USA). Relative injury (RI)

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which represented electrolyte leakage or cell membrane integrity was estimated by the following equation:

$$RI\ (\%) = 1 - [\{1/(T_1/T_2)\}/\{1 - (C_1/C_2)\}] \times 100,$$

where T and C referred to conductance for treatment and control vials, respectively and subscripts 1 and 2 are initial and final conductance, respectively. Seed yield was recorded after the crop harvest. Test of significance (P<0.05 and 0.01) and relationships among different characteristics were made by using Online Statistical Analysis Package (OPSTAT).

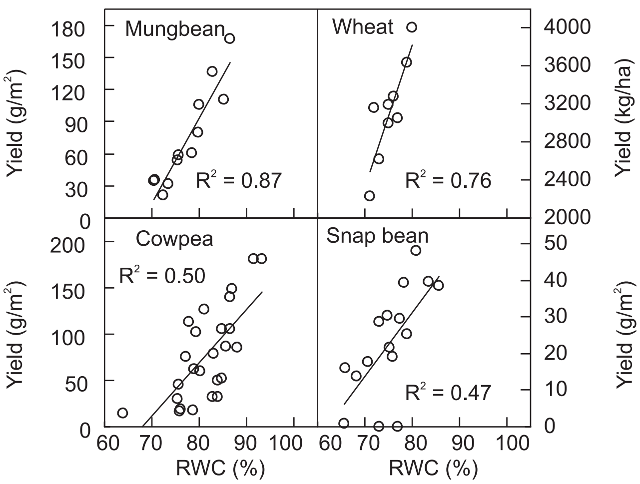

Fig 1 Relationship of seed yield with leaf relative water content (RWC) in mung bean (n= 12, $^{**}P < 0.01$), wheat (n = 9, $^{**}P < 0.01$), cowpea (n = 25, $^{**}P < 0.01$) and snap bean (n = 16, $^{*}P < 0.05$). RWC was measured in the youngest fully expanded leaf in mung bean, cowpea and snap bean during pod formation and in flag leaf in wheat at anthesis between 1200–1400 hr.

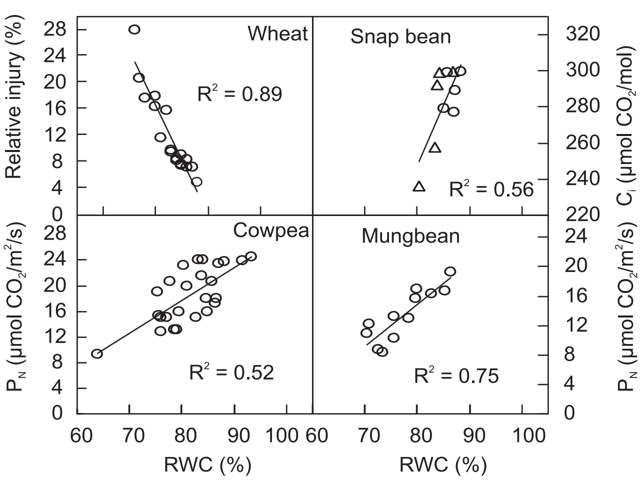

Fig 2 Relationship of leaf relative water content (RWC) with relative injury (RI) in wheat, intercellular CO_2 concentration (C_i) in snap bean and rates of photosynthesis (P_N) in cowpea and mungbean. The physiological characteristics were measured in the youngest fully expanded leaf in mungbean, cowpea and snap bean during pod formation and in flag leaf in wheat at anthesis between 1200 and 1400 hr $^{**}P < 0.01$.

The results showed that leaf water content (RWC) measured during midday hours showed distinct genotypic variation in youngest fully expanded leaf in snap bean, cowpea and mungbean during pod formation and in flag leaf during anthesis in wheat. RWC was positively associated with seed yield in the 4 crops, cowpea, mungbean, snap bean and wheat (Fig 1). In each crop, cultivars maintaining higher RWC were more productive and *vice versa*. More complex parameters such as cellular membrane integrity (measured as relative injury to cell membrane), rates of photosynthesis (P_N) and intercellular CO_2 concentration (C_i) were associated with RWC. Relative injury was negatively and C_i and P_N were positively correlated with RWC (Fig 2) indicating that plants displaying high RWC allowed more CO_2 entry in the cells resulting into high P_N as well as low leakage of photosynthetic products due to greater hydration of cell membranes. Therefore, leaf water content is an important physiological trait for improved productivity, and it can be used as a screening tool for drought tolerance in field crops.

SUMMARY

The role of physiological understanding from the perspective of breeding programmes was examined in many crops and it was found that RWC measured during midday hours (1200 – 1400 hr) was a good indicator of plant performance under drought conditions. RWC in the youngest fully expanded leaf of cowpea (*Vigna unguiculata* L. Walp.), mungbean (*Vigna radiata* L.) and snap bean (*Phaseolus vulgaris* L.) estimated during pod formation and in flag leaf of wheat (*Triticum aestivum* L.) during anthesis was positively correlated with seed yield. Complex parameters such as cellular membrane integrity, intercellular CO_2 concentration and rates of photosynthesis were significantly correlated with RWC. Therefore, leaf water content expressed as RWC is an effective indicator of plant performance under drought conditions, and it has utility to be used as selection criteria in plant breeding programmes for screening crop germplasm for drought tolerance.

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