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Water Stress Affected Seed Accumulation of Non-reducing Soluble Sugars and Germination Performance of Three Chickpea Cultivars

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ABSTRACT

Background: Water stress may alter the seed chemical composition and cause changes in the concentrations of non reducing soluble sugars in seeds and germination performance of chickpea. We evaluated the effect of water stress during chickpea seed development on seed (i) accumulation of non-reducing soluble sugars, and (ii) vigour and germination.

Methods: Three chickpea cultivars (Desi-K, Saina-K and ICCV-K) were subjected to two water regimes (water stress- withholding irrigation after flowering and non-stressed- irrigation at 3-day intervals throughout the crop growth) in a completely randomized design with three replications. Seed size, viability, sugars content, solute leakage, germination percentage and mean germination time were determined from seeds harvested at physiological maturity.

Result: Water stress increased the raffinose seed content of Saina-K and Desi-K, decreased the raffinose seed content in ICCV-K, but had no effect on sucrose and stachyose content. Electrical conductivity (EC) increased with water stress in all cultivars, but the increase was greater in ICCV-K (57%) compared to Desi-K (18%) and Saina-K (12%). Non-stressed seeds had larger seed size, higher seed viability and germination percentage and lower EC and mean germination time than stressed seeds. Clearly, sufficient water at seed development stage is crucial for productivity of subsequent crops as it affects seed quality. However, we recommend further studies using a wide range of water stress treatments and chickpea cultivars with different seed size, seed color and seed coat texture.

Key words: Legumes, RFOs, Seed size, Seed viability, Seed vigour, Watering regimes.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop that plays a significant role in addressing global food insecurity challenges (Jha *et al.,* 2021). It is an excellent colonizer of low nitrogen soils (Liu *et al.,* 2020), an alternative source of cheap protein and does well in relatively poor soils (Semba *et al.,* 2021). Chickpea is mainly grown in developing countries, accounting for 95% of the world's production, with India (65%) the leading producer (FAO, 2019). There is limited chickpea production in South Africa despite a huge and rising domestic demand (Mpai and Maseko 2018). Several biotic and abiotic factors limit chickpea productivity, with water stress being one of the most critical because it reduces both grain yield (Ullah *et al.,* 2020) and seed quality (Begna, 2020). Water stress during seed development reduces carbon dioxide assimilation, thus limiting the production of assimilates necessary for the synthesis of soluble carbohydrates, which are essential sources of energy for germination and crop establishment (Siddique *et al.,* 2016; Kapoor *et al.,* 2020). Moreover, a strong relationship exists between water stress tolerance and the concentration of oligosaccharides (ElSayed *et al*., 2014).

Raffinose, stachyose and verbascose (which belong to the raffinose family of oligosaccharides, RFOs) and sucrose, which are the major non-reducing soluble sugars in legumes (Gangola *et al.,* 2013), play a significant role in the acquisition of desiccation tolerance, which protects seeds against cell membrane and cytoplasm disruption (Guimaraes *et al.,* 2016). They accumulate in orthodox seeds during the acquisition of desiccation tolerance and are degraded when the tolerance is lost (Valentine *et al.,* 2017; Vertucci and Farrant, 2017). Although some studies (e.g., Lahuta *et al.,* 2000) have demonstrated a relationship between RFOs accumulation and desiccation tolerance under water stress conditions in legume species, there is

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hardly any documented information on the effect of this relationship on seed quality. Moreover, little is known about the effect of water stress during seed development on the concentrations of soluble carbohydrates in the seed and how this may affect seed vigour. Siddique and Wright (2004) suggested that differences in environmental conditions, the length of the seed filling period and concentrations of soluble carbohydrates could cause variation in the seed vigour of peas, but none of these variables could solely explain the observed variations in seed vigour. Besides, it is not known whether the accumulation of RFOs in response to water stress may vary with chickpea genotypes.

Therefore it was hypothesised that water stress during seed development would cause variable alterations in accumulation of soluble sugars and consequently germination and seed vigour of different chickpea cultivars. The hypothesis was based on the fact that these compounds are implicated in the acquisition of desiccation tolerance and changes in their content in seeds may affect cell membrane integrity and consequently germination and seed vigour of chickpea. The objective of the study was to assess the effect of water stress during seed development on (i) the final content of seed soluble sugars (sucrose, raffinose and stachyose), and (ii) germination and seed vigour of three chickpea cultivars.

MATERIALS AND METHODS

A pot experiment was carried out at the Controlled Research Facility Centre (Polytunel, where temperature, light and relative humidity were not regulated but the plants were sheltered from rainfall), University of KwaZulu-Natal, Pietermaritzburg (29° 37′30° 24′596), South Africa in 2016 and 2017. Three chickpea cultivars sourced from Kenya, 1 desi (Desi-K) and 2 *kabuli* (Saina-K and ICCV-K), were subjected to two water regimes (stressed - withholding irrigation after flowering and non-stressed - irrigation with 800 mL pot^{-1} water 3 times a week, which maintained the soils at field capacity, throughout the crop growth) using a completely randomized design with three replications. The stressed plants were subjected to water potentials greater than -400 kPa (Odindo, 2007). Each treatment combination had ten 14L pots, giving a total of 180 experimental units. The seeds were harvested at physiological maturity and used for the determination of seed size, sugars content, solute leakage, germination percentage (GP) and mean germination time (MGT).

Four replicates of forty seeds each was used to determine seed size, viability and solute leakage. Seed viability and size were determined using the tetrazolium chloride (TZ) test (ISTA, 2012) and a vernier calliper (OMNI-TKCH®), respectively. Seeds were immersed in distilled water for 18 hours at room temperature (±21°C) before being cut longitudinally and thereafter put in a 90 mm petri dish and fully immersed in TZ solution for 2 hours. Tissues that stained reddish pink were regarded as viable, while those unstained were considered unviable. The number of viable seeds was counted from each treatment and the seed viability was calculated using equation 1.

$$\text{Seed viability (\%)} = \left[\frac{\text{Number of stainedembryos}}{\text{Total number of embryos}}\right] \times 100 \quad \text{...(1)}$$

To determine solute leakage from seeds, the electrical conductivity (EC) of seeds was measured using the EC meter (Jenway, 4510 model) according to ISTA (2012). Ten seeds from each experimental unit were put into 80 mL beakers and immersed in 20 mL of distilled water for 24 hours after which EC was recorded from the imbibed seeds solution. Equation 2 was used to calculate the conductivity.

$$\text{EC } (\mu\text{S cm}^{-1}\text{ g}^{-1}) = \frac{\text{Leachate conductivity - Blank conductivity}}{\text{mass of 10 seeds}} \quad \text{...(2)}$$

Where,

leachate conductivity was the EC of soaked seeds solution and the blank conductivity was the EC of a clear prism/ ultra-pure water used to soak the seeds.

The GP was determined from four replicates of forty seeds each using the moist brown paper towel method (ISTA, 2012). Germination was evaluated by counting, from day 0 to day 8, the number of germinated seeds that had 2 mm radicle protrusion (ISTA, 2012). Equation 3 was used to calculate the GP.

$$\text{GP} = \left(\frac{\text{Seeds germinated}}{\text{Total seeds}}\right) \times 100 \quad \text{...(3)}$$

MGT was assessed daily from the day of planting (Day 0) until there was no increase in number of germinated seeds. The data obtained from the assessment was used to calculate MGT using equation 4 (Heydecker, 1968).

$$\text{MGT} = \left[\frac{\Sigma FX}{\Sigma X}\right] \quad \text{...(4)}$$

Where

F= Number of days from the beginning of the germination test.

X= Number of newly germinated seeds on that day.

A sample of 120 seeds was ground into a fine powder for the determination of the soluble sugars as described by Tesfay and Magwaza (2017), with slight modification. The sugars were extracted from 0.5 g of seed powder using 80% v/v methanol (10 mL). Concentrations of stachyose, raffinose and sucrose were determined using a HPLC binary pump system (Agilent Technologies, UK). Sample extracts were injected into a Rezex RCM monosaccharide Ca+ (8%) column of 7.8 mm × 300 mm (Phenomenex, Torrance, CA, USA). The column temperature was set at 86°C using a column compartment (G1316A, Agilent). The presence and concentration of the selected sugars were calculated by comparing the peak area of samples against the peak area of known standard concentrations using equations from standard curves (0.05-1.25 mg/mL; R^2 = 0.995) (Tesfay and Magwaza, 2017).

The data for the two years were pooled and subjected to two-way analysis of variance using GenStat® software (18th edition). Significant differences between the treatment means were compared using the least significant difference ($P \leq 0.05$) test.

RESULTS AND DISCUSSION

Physiological seed quality traits

The interaction between water regime (WR) and cultivar (C) affected seed size, EC and MGT but had no effect on seed viability and GP (Table 1). However, the main effects of WR and cultivar on seed viability and germination were significant (Table 1). Water stress reduced the seed size of Saina-K (by 12%) and Desi-K (by 10%) but did not affect the seed size of ICCV-K (Fig 1a). The EC increased with water stress in all cultivars, but the increase was greater in ICCV-K (57%) compared to Desi-K (18%) and Saina-K (12%) (Fig 1b). In contrast, water stress increased the MGT of Desi-K and Saina-K recorded the lowest MGT at both watering regimes (Fig 1c). The increase in EC under water stress indicates high solute leakage which is associated with the presence of dead tissues within the seeds that result from the loss of cell membrane integrity (Ebone *et al*., 2019; Bakhshandeh and Jamali, 2020). The loss of cell membrane integrity due to water stress has been associated with poor seed development and performance during germination (Bakhshandeh and Jamali, 2020) which is consistent with our findings as evidenced by the lower seed size and seed viability and the higher MGT with water stress (Table 1).

The cell membrane loses its integrity as seeds dry out at maturity and is re-established during imbibition (Zhang *et al.,* 2021). However, the re-establishment of these membranes is much faster on vigorous seeds with low leakage than on less vigorous seeds with high leakage. This may partly explain the variable effect of water stress on EC and MGT of the different chickpea cultivars observed in the current study. For example, Saina-K which exhibited the lowest solute leakage under water stress conditions also recorded the lowest MGT under both water regimes and the highest GP averaged across the water regimes compared to the other cultivars. The differential response of seed quality of the three cultivars to water stress we observed could also be attributed partly to differences in seed coat adherence and hence imbibition damage (Llanes *et al.,* 2016). Vilakazi (2018) observed that ICCV-K that had a more loosely attached seed coat to the cotyledon, also showed more rapid and higher imbibition compared to the other genotypes under both water regimes and this was reflected in the higher solute leakage and MGT in ICCV-K compared to Saina-K, coupled with highest average MGT across the water regimes in the current study. Water stress reduces seed fill duration during dry matter accumulation (Monzon *et al.,* 2021) and hence the observed decrease in seed size under water stress conditions in the current study was not unexpected. However, it is not clear why this reduction in seed size did not appear to have any association with membrane permeability and germination performance in the current study and this requires further investigation.

Table 1: The response of chickpea seed quality to water stress during seed development.

Treatments	Morphological and Physiological Parameters				
	Seed size (mm^2)	Electrical conductivity ($\mu S\ cm^{-1}\ g^{-1}$)	Seed viability (%)	Germination (%)	Mean germination time (day)
Water regime (WR)					
Non-stressed	45.31^a	648.02^b	74.24^a	63.32^a	1.26^b
Stressed	41.90^b	833.33^a	55.81^b	60.01^a	1.82^a
LSD	1.462	36.404	11.043	15.651	0.348
Cultivar (C)					
Saina-K	45.14^b	579.33^c	71.21^a	77.53^a	1.22^b
Desi- K	38.60^c	779.82^b	65.03^a	58.81^b	1.69^a
ICCV- K	47.21^a	863.04^a	58.80^a	48.80^b	1.71^a
LSD	1.792	44.581	13.530	19.163	0.432
P (f-ratio)					
WR	**	**	*	ns	*
C	**	**	ns	*	*
WR × C	*	**	ns	ns	*
CV (%)	2.1	2.9	2.1	11.5	8.8

** Highly significant ($P<0.001$), significant * ($P<0.05$),and ns (not significant at $P>0.05$).

Means in the same column followed by the same letter are not significantly different, and least significant difference of the means (LSD); coefficient of variation (CV).

The accumulation of soluble sugars

Water stress increased raffinose content (by 5.9%) but had no significant effect on sucrose and stachyose content (Table 2) which suggests that the RFOs plays a role in stress adaptation (Valentine *et al*., 2017). Moreover, raffinose content was subject to WR x C interaction; water stress increased the raffinose content of Saina-K by 10% but had no significant effect on the raffinose content of Desi-K and ICCV-K (Fig 1d). Previous studies show that raffinose accumulation plays a significant role in the acquisition of desiccation tolerance and hence protects seeds against disruption of both the cell membrane and cytoplasm during stress, which results in higher seed germination and vigour

Table 2: The effect of water stress during seed development on the accumulation of sugars in chickpea seed.

Treatments	Non-reducing soluble sugars		
	Raffinose (µg g^{-1})	Sucrose (µg g^{-1})	Stachyose (µg g^{-1})
Water regime (WR)			
Non-stressed	56.31^{b}	74.31^{a}	74.23^{a}
Stressed	59.61^{a}	74.40^{a}	76.34^{a}
LSD	3.100	5.050	5.321
Cultivar (C)			
Saina-K	63.22^{a}	81.21^{a}	76.14^{a}
Desi- K	58.20^{b}	74.90^{b}	77.53^{a}
ICCV- K	52.46^{c}	66.93^{b}	72.10^{a}
LSD	3.797	6.182	6.520
P (f-ratio)			
WR	*	ns	ns
C	**	**	ns
WR × C	*	ns	ns
CV (%)	7.6	9.9	8.6

** Highly significant (P<0.001), significant * (P<0.05) and ns (not significant at P>0.05).

Means in the same column followed by the same letter are not significantly different and least significant difference of the means (LSD); coefficient of variation (CV).

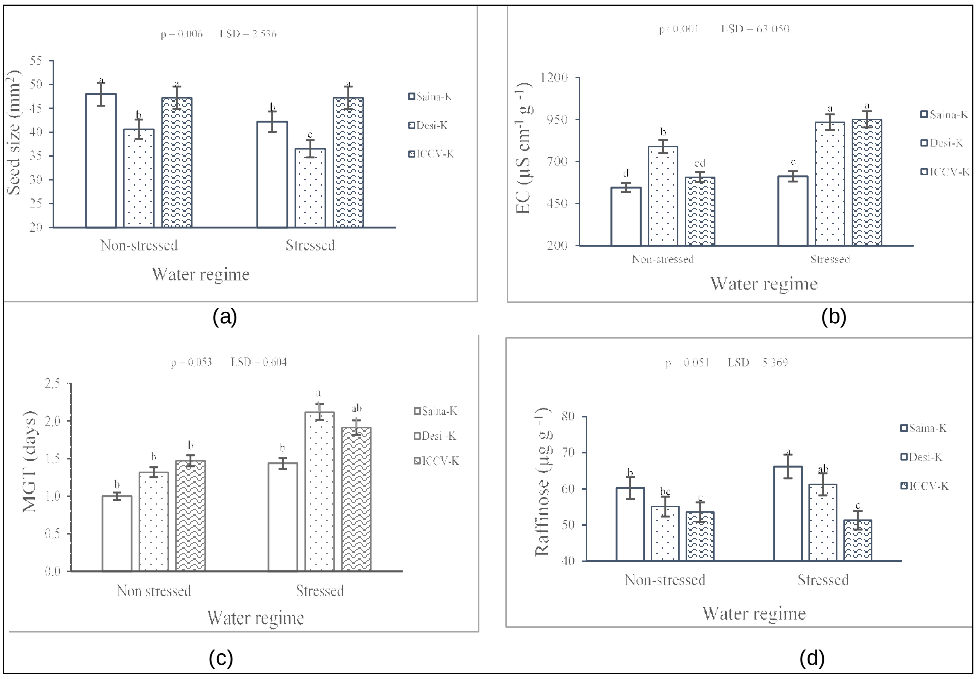

Fig 1: The interactive effect of water regime and cultivar on (A) size, (B) electrical conductivity, (C) mean germination time, and (D) the raffinose content of chickpea seed.

(Jing *et al*., 2018). Saina-K recorded the highest raffinose content, and exhibited lowest solute leakage and MGT compared to the other genotypes under water stress which is consistent with the findings of Jing *et al*. (2018).

The positive relationship between raffinose content and seed quality is partly because raffinose is an important source of energy for germination and crop establishment (Siddique *et al.,* 2016). However, enhanced production of raffinose in response to the dehydration signal might be a preparation to maximize future longevity of the dried embryo rather than to initiate tolerance to desiccation (Matilla*,* 2021). Therefore, the assumption that raffinose and other oligosaccharides play equivalent roles in the acquisition of desiccation tolerance, storability, and longevity needs further investigation in chickpea and other pulses.

The variation in sucrose content with cultivars which followed a similar pattern to the variation in raffinose content, with Saina-K and ICCV-K recording the highest and lowest levels of both oligosaccharides, respectively (Table 2), was not unexpected because sucrose acts as a galactosyl acceptor in RFOs biosynthesis (Saldivar *et al.,* 2011; Gangola *et al.,* 2016). However, sucrose accumulates independently of desiccation tolerance while raffinose accumulation is dependent on desiccation tolerance of seeds (Zhang *et al.,* 2016). This probably explains the non-significant effect of water stress on sucrose content observed in this study.

CONCLUSION

Water stress during chickpea seed development caused alteration in the seeds' non-reducing soluble sugars, which consequently affected seed germination. Moreover, the effect of water stress on solute leakage, raffinose content and MGT varied with genotype with Saina-K showing superior performance in all the the traits compared to the other genotypes. Clearly, water stress affects chickpea seed quality through its effect on membrane stability and accumulation of raffinose in the seed but this effect varies with chickpea genotype. Therefore, we recommend further investigations using a wide range of water stress treatments and chickpea genotypes chracterised by varying seed size, seed colour and texture of seed coat.

Conflict of interest: None.

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