Field Crops Research 168 (2014) 1–7

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

The critical period for yield determination in chickpea (*Cicer arietinum* L.)

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ARTICLE INFO

Article history:
Received 8 May 2014
Received in revised form 8 August 2014
Accepted 8 August 2014
Available online 3 September 2014

Keywords:
Yield determination
Critical period
Yield components
Stress
Seed number
Seed size

ABSTRACT

Chickpea seed yield is highly variable as a result of biotic, mostly fungal, and abiotic stresses including extreme temperatures and water stress. The effect of stress on yield depends on its intensity, timing and duration, hence the importance of knowing the critical window of yield formation and stress vulnerability. This window has not been determined in chickpea. To fill this gap, we compared the effect of sequential 14-d shading periods on the yield and yield components of two chickpea varieties, PBA Boundary and PBA Slasher, in three environments where unshaded controls yielded between 2880 and 3130 kg ha^{-1}. Unlike other species which do not respond to stress early in the season, shading reduced yield from emergence until the beginning of the critical period, 300 °Cd before flowering (base temperature = 0 °C). The critical period was found to be at least 800 °Cd long centred 100 °Cd after flowering. Seed number accounted for most of the variation in yield, which was unrelated to seed size. Pod number accounted for most of the variation in seed number prior to the critical period, while pod number and seeds per pod contributed to seed number within the critical period. After 400 °Cd post flowering, seeds per pod was the main variable affecting seed number. This information can be used in breeding and agronomy to improve stress adaptation.

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1. Introduction

Chickpea (*Cicer arietinum* L.) is one of the most important grain legumes worldwide (Berger et al., 2006; Krishnamurthy et al., 2013). It is grown predominantly in south Asian and Mediterranean environments; India is the largest producer with 7.7 million tonnes in 2012 (FAO, 2013). In Australia, production increased from 129,000 t in 2002 to 673,000 t in 2012 (FAO, 2013) making it the second largest world producer in 2012. Chickpea yield is constrained by biotic stresses, particularly fungal diseases such as *Ascochyta* blight (*Ascochyta rabiei*) (Knights and Siddique, 2003) and abiotic stresses such as water deficit and extreme temperatures (Knights and Siddique, 2003; Kashiwagi et al., 2006; Leport et al., 2006). As a result of poor adaptation to these stresses, chickpea can be perceived as relatively unstable and low yielding (Millan et al., 2006).

The effect of abiotic stresses on crop yield depends on the intensity, timing and duration of the stress, hence the effort to determine the critical period underpinning yield determination in major crops. Species specific critical periods have been determined for cereals; wheat, barley, triticale and maize (Fischer, 1985; Kiniry and Ritchie, 1985; Savin and Slafer, 1991; Arisnabarreta and Miralles, 2008; Estrada-Campuzano et al., 2008; Cerrudo et al., 2013), sunflower (Cantagallo et al., 1997) and the grain legumes; soybean, peas and lupin (Board and Tan, 1995; Jiang and Egli, 1995; Guilioni et al., 2003; Sandaña et al., 2009; Sandaña and Calderini, 2012). Identification of critical periods aids in crop breeding and management, and ultimately improved yield and yield reliability (Sandaña and Calderini, 2012; Cerrudo et al., 2013).

In cereals the critical period has been commonly identified around the stage leading up to anthesis in barley (Arisnabarreta and Miralles, 2008), has extended into flowering for wheat and triticale (Fischer and Stockman, 1980; Fischer, 1985; Estrada-Campuzano et al., 2008), and even further post anthesis for maize (Cerrudo et al., 2013). In grain legumes, the majority of the critical period occurs further into seed filling with soybean identified as R1 (beginning of flowering) to R5 (beginning of seed set) and 10 days before R1–R5 for lupin and field pea (Board and Tan, 1995; Jiang and Egli, 1995; Sandaña and Calderini, 2012). The most likely reason for grain legume critical periods extending into seed filling is overlapping vegetative and reproductive stages and continuation of flowering after seed set (Slafer et al., 2009).

The most common method to determine the critical period is the application of shade to cause source reduction at different developmental stages (Fischer, 1985; Savin and Slafer, 1991; Jiang and Egli,

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http://dx.doi.org/10.1016/j.fcr.2014.08.003
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1993; Arisnabarreta and Miralles, 2008; Estrada-Campuzano et al., 2008; Sandaña et al., 2009; Sandaña and Calderini, 2012; Cerrudo et al., 2013). Shading is highly repeatable, and affects crop growth rate, which is correlated with seed set in the critical period (Fischer, 1985; Egli and Yu, 1991; Jiang and Egli, 1995; Andrade et al., 2005; Arisnabarreta and Miralles, 2008; Kantolic et al., 2013). Defoliation has also been used to cause source reduction (Board and Harville, 1993; Board and Tan, 1995) but may produce undesirable and confounded effects, such as soil temperature or moisture differences caused by reduced canopy. Defoliation is also likely to have effects associated with the removal of stored nitrogen from vegetative organs (Munier-Jolain et al., 1998; Lhuillier-Soundélé et al., 1999; Sandaña et al., 2009) and may also result in unintended and confounding competition effects. Munier-Jolain et al. (1998) reported no difference between the seed number of defoliated and control plants but a significant reduction in seed number of shaded plants compared to controls. Bertero and Ruiz (2008) used an indirect method to determine critical period in quinoa, looking at the association between crop growth rate in specific phenophases and seed number; however they recognise the need to enhance these results using shading.

In previous studies, sequential periods of shading have caused an increasing reduction in grain number as the critical period approaches, with little effect on grain weight (Fischer, 1985; Arisnabarreta and Miralles, 2008; Estrada-Campuzano et al., 2008; Sandaña and Calderini, 2012). The reductions in grain number generally resulted from less fertile florets per spike in cereals or reduced pod number in legumes.

Currently there is limited information on the critical period for yield determination in grain legumes and no information in chickpea. The aim of this study was to determine the critical period for yield determination in chickpea.

2. Methods

2.1. Plant material, environments and experimental design

Two chickpea varieties were grown in three environments. Varieties PBA Slasher and PBA Boundary were selected on the basis of reported phenotypic traits. PBA Slasher is adapted to Southern and Western Australian chickpea growing regions, is mid flowering and maturing, is Ascochyta blight resistant and is semi spreading. PBA Boundary is adapted to Northern New South Wales and Southern Queensland chickpea growing regions, is mid maturing, is Ascochyta blight resistant and has a tall erect plant type. Actual differences between varieties in key traits including development and yield were smaller than expected under our experimental conditions (Section 3). The three environments resulted from combinations of locations and sowing dates: Roseworthy (34°52′S, 138°69′E) sown on 7th June, Turretfield (34°33′S, 138°49′E) at recommended sowing date (14th June – TOS 1) and Turretfield late sown (9th of July – TOS 2). Roseworthy was supplied with 12 mm of supplemental irrigation at flowering. Daily weather data was obtained from the Roseworthy and Turretfield weather stations from the Queensland Government, Long Paddock website (http://www.longpaddock.qld.gov.au/silo/). Thermal time was calculated from daily mean temperature using a base temperature of 0 °C (Berger et al., 2006).

Crops were sown after barley in a Calcic Luvisol (http://www.fao.org/fileadmin/user_upload/soils/docs/Soil_map_FAOUNESCO/new_maps/X_1_petit.jpg) at Roseworthy, and after canola into Calcic Luvisol at Turretfield. The target plant density was 50 plants m^{-2}. The seed was pre-treated with P – Pickel T fungicide to minimise the risk of seed borne *Ascochyta* blight and inoculated with Group N rhizobia immediately before sowing. For all other seed treatments, fertiliser, insect, disease and weed management, agronomic practices were carried out in accordance with the protocols of the National Variety Trials (http://www.nvtonline.com.au/).

A split-plot design with four replicates was used where varieties were allocated to main plots and shading treatments, including unshaded controls, to randomised subplots. Plot size was 29 m^{-2}, comprised of 6 rows (spaced 24.2 cm) of 20 metres length. Shading treatments lasted for 14 days each, and were designated sequentially from 1 to 8, starting at 31 days (353 °Cd) after sowing at Roseworthy and 24 days (251 °Cd) after sowing at Turretfield TOS 1. Turretfield TOS 2 had a shorter growing season and had six shading treatments in sequence beginning 35 days (399 °Cd) after sowing. Owing to an error in shade placement, data from Turretfield TOS 1 shading treatment number 1 was discarded. Shading was ceased when plants within the experimental plots had ceased flowering, pods had yellowed and were perceived to have reached the final stage in seed abortion where no more yield loss was anticipated (Ney and Turc, 1993; Munier-Jolain et al., 1998). Plants were then harvested when completely desiccated several weeks later. The shades were constructed from black shade cloth that intercepted 90% of solar radiation and were maintained at a minimum of 10 cm from the top of the canopy at all times. The shade cloth was constructed into a frame using wire and wooden stakes so that plants were shaded from the top and the sides, with the southern side left open to allow for regular temperature variation and air movement. The size of the shaded area was 1.1 m × 1.1 m (1.21 m^2) with five of the six rows being shaded.

2.2. Traits

Weekly phenology observations were used to determine time of first flower (FF), fifty percent flowering (50F), pod emergence (PE), when 50% of plants showed visible pods, and end of flowering (EOF), when 50% of plants ceased flowering (Berger et al., 2004). Maturity was scored when 50% of pods in a plot had matured. Flowering duration was calculated as the time from 50% flowering to end of flowering. Phenological stages are expressed on a thermal time scale.

Yield and yield components were measured at maturity from samples taken from 3 m × 0.5 m length cuts of central rows of the shaded area; border rows were excluded (Rebetzke et al., 2014). Yield components included pod number, pod weight, seed size, seeds per pod, shoot biomass and the derived traits pod wall ratio (PWR = pod wall weight/whole pod weight (Lagunes-Espinoza et al., 1999; Clements et al., 2005; Sadras et al., 2013)) and harvest index (HI = seed yield/shoot biomass).

2.3. Data analyses

The effect of timing of shading, variety and the interaction was tested using analysis of variance separately for each environment as there was unequal numbers of shading treatments among environments. Fisher's PSLD test was used to determine differences between timing of shading treatment and unshaded controls.

Yield and yield components in shading treatments were normalised as a fraction of the control, and the trajectory of normalised traits was plotted against the phenology of controls (thermal time scale centred at flowering); curves were fitted by eye, as it has been done previously (Arisnabarreta and Miralles, 2008; Estrada-Campuzano et al., 2008; Sandaña and Calderini, 2012).

3. Results

3.1. Environmental conditions and crop development

Weather between sowing and flowering was very similar between environments with small differences reflecting the

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