Chapter 39
The Effect of Earliness per se (*Eps*) Genes on Flowering Time in Bread Wheat

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Abstract Photoperiod (day-length) response, vernalization (response to extended periods of cold) and earliness per se (*Eps*) genes regulate flowering time in wheat. The vernalization and photoperiod response genes are relatively well studied. However, the role of *Eps* genes is yet to be fully understood but the current assumption is that *Eps* genes regulate flowering independent of vernalization and photoperiod. While some *Eps* genes have been cloned in both *Hordeum vulgare* and *Triticum monococcum*, none has been cloned in *Triticum aestivum* to date. The use of near isogenic lines (NILs) in both *T. monococcum* and *Triticum aestivum* has enabled *Eps* effects to be studied in more detail and candidate genes have been proposed for *Eps* effects in both species. *Eps* loci are reported to be involved in fine tuning flowering time and are also responsible for controlling spikelet number and size hence could be manipulated to increase wheat yield. This mini review summarises our current understanding of *Eps* and how manipulation of *Eps* genes can be used in predictive wheat breeding.

The world population demands more food, greater diversity of food, a balanced and healthy diet, produced on no more, and preferably less land, while conserving soil, water, and genetic resources. The major problem is that even though wheat yields are increasing (Lopes et al. 2012), the percentage increase is below the projected percentage demand with about 0.6 % deficit projected annually until 2050 (Dixon et al. 2009; Rosegrant and Agcaoili 2010). The challenge wheat breeders face is to bridge the gap between wheat demand and wheat production. It is therefore vital to direct wheat breeding efforts to the production of higher yielding varieties in order to ensure current and future food security (Reyolds et al. 2012). The part of the wheat plant that is important for direct consumption by humans is the grain and its production is dependent on flowering time. Manipulating flowering time is one avenue that can be exploited to increase wheat grain yield (Herndl et al. 2008; Greenup et al. 2009). However, in order to successfully increase grain yield, it is

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vital to thoroughly understand the genetic and physiological factors affecting wheat growth and development particularly flowering time genes (Gill et al. 2004).

There are three major classes of flowering time genes which are photoperiod (*Ppd*) response genes, vernalization (*Vrn*) response genes and earliness per se genes (*Eps*). Major photoperiod response genes enable wheat plants to perceive changes in day length with accelerated flowering occurring in long days while short days cause delayed flowering unless there are mutations in the *PHOTOPERIOD 1* (*Ppd-1*) genes (Beales et al. 2007; Wilhelm et al. 2009; Dı'az et al. 2012). Three main genes *VERNALIZATION 1*, *2* and *3* (*VRN1*, *VRN2* and *VRN3*) control the vernalization response in wheat (Yan et al. 2003; Trevaskis et al. 2007; Distelfeld et al. 2009a, b; Shimada et al. 2009; Distelfeld and Dubcovsky 2010; Dı'az et al. 2012). Wild type wheat require extended exposure to cold (known as winter wheat) before the transition from vegetative to reproductive growth while mutants do not require this exposure and are regarded as spring wheat (Fu et al. 2005).

The third class of genes controlling flowering time is earliness per se, also referred to as ear emergence per se, earliness in narrow sense, intrinsic earliness, and at times is called basic development rate (Laurie et al. 2004; Cockram et al. 2007; Shitsukawa et al. 2007; Lewis et al. 2008). A number of similar definitions have been proposed for *Eps*. *Eps* can be defined as the minimum number of days to reproductive growth, after vernalization and photoperiod requirements are satisfied (van Beem et al. 2005). Similarly, Appendino et al. (2003) defined *Eps* as the time to heading after both vernalization and photoperiod requirements are satisfied. Shitsukawa et al. (2007) defined narrow sense earliness or earliness per se as the earliness of fully vernalized plants grown under long days. Lewis et al. (2008) described *Eps* as all other genes controlling flowering time but not involved in either vernalization or photoperiod requirements. The *Eps* definitions suggest that these genes regulate flowering independent of both vernalization or photoperiod environmental cues (Bullrich et al. 2002).

The course and fine adjustment knobs of a light microscope can be used to visualise the role of the *Eps* genes in flowering time (Fig. 39.1). The *Ppd* and *Vrn* genes would be equivalent to the course adjustment knob and are responsible for adaptation to mega environments for example spring and winter wheat as well as short day and long day environments (Worland et al. 1994, 1998). The *Eps* genes are equivalent to the fine adjustment knob (Fig. 39.1) and are responsible for fine-tuning of wheat flowering time (Valarik et al. 2006) within mega-environments (Griffiths et al. 2009) and are responsible for wide adaptation of wheat to different environments (Lewis et al. 2008). Laurie et al. (2004) suggested that *Eps* factors may be largely responsible for the variation in flowering time in crosses within winter or spring types provided they have the same alleles at the major photoperiod and vernalization response loci.

Earliness per se causes differences of a few days in flowering time under field conditions (Valarik et al. 2006; Griffiths et al. 2009; Zikhali et al. 2014). In *Triticcum monococcum*, it has been shown that while the *Eps* effect on chromosome 1A designated *Eps-A^{m}1* causes flowering differences of only a few days at 23 °C, this difference increased to several weeks when the plants were fully vernalized and grown under long days at 16 °C (Appendino and Slafer 2003). In a recent study,

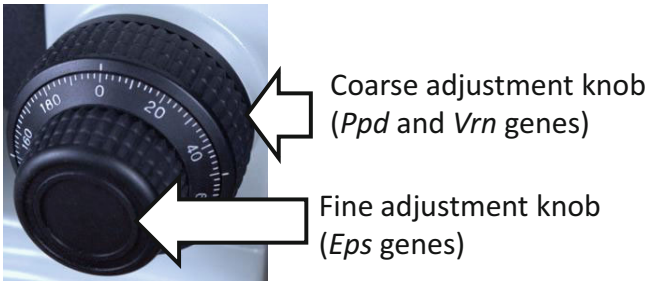

Fig. 39.1 Schematic presentation, using the fine and coarse adjustment knob of the light microscope, of the role of *Eps* genes in flowering time. The coarse adjustment knob represents the role of photoperiod (*Ppd*) and vernalization (*Vrn*) genes in influencing mega environment adaptation while the *Eps* genes adapt flowering within mega environments. Understanding *Eps* genes will enable their manipulation and fine-tuning of flowering time which may enable precision breeding in wheat

Zikhali et al. (2014) it was shown that cultivar Rialto flowers more than 12 days earlier than cultivar Spark in Short days but when grown for 8 weeks under short days and then moved to long days, the *Eps* effect on chromosome 1DL causes Spark to flower 5 days earlier. This result shows that while the overall difference in flowering time is 5 days, the *Eps* effect in Spark also overcomes the earliness conferred on Rialto by the short days prior to moving into the long days. Earliness per se is often considered polygenic (Rousset et al. 2011). Determining the role played by the individual *Eps* genes in each developmental phase may enable breeders to fine tune ear emergence in predictive wheat breeding (Griffiths et al. 2009) and increase wheat yield in different environments (Lewis et al. 2008). To determine the role of an individual *Eps* gene, on different wheat developmental phases requires knowing what the gene is and hence the need for accurate mapping of the gene responsible (Lewis et al. 2008).

Because of their relatively small effect, *Eps* genes were previously mapped only as QTLs in wheat (Miura et al. 1999). However, *Eps* genes have been defined more accurately in the recent years using near isogenic lines (NILs). One *Eps* gene that has been well defined after almost a decade of study is the *Eps-A^{m}1* reported to be on the distal region of *T. monococcum* chromosome 1A^{m}L (Bullrich et al. 2002; Valarik et al. 2006; Faricelli et al. 2010). The gene has been recently reported to be involved in determining the number of spikelets as well as the number of grains per spike in diploid wheat in addition to affecting heading time (Lewis et al. 2008). The genes *MOLYBDENUM TRANSPORTER 1* (*MOT1*) and *FILAMENTATION TEMPERATURE SENSITIVE H* (*FtsH4*) are the suggested candidates for the *Eps-Am1* (Faricelli et al. 2010) although work is in progress to definitively identify the gene responsible. The *Eps-3Am* locus has also been well defined (Mizuno et al. 2012; Gawroński et al. 2014). The *Eps-3Am* QTL interval in *T. monococcum* was fine mapped using high-density mapping (Gawrosnski and Schnurbusch 2012). A recent report suggested a *T. monococcum* ortholog of the *Arabidopsis thaliana LUX ARRHYTHMO/PHYTOCLOCK 1* (*LUX/PCL1*) as a potential candidate of the

Eps-3Am which was suggested to act by distorting the circadian clock (Gawroński et al. 2014).

There are some striking similarities between *Eps-Am1* and *Eps-3Am.* Both *Eps-Am1* and *Eps-3Am* loci were reported to determine the number of spikelets as well as the number of grains per spike in addition to affecting heading time (Lewis et al. 2008; Gawroński et al. 2014). Again both *Eps-Am1* and *Eps-3Am* have been reported to be thermosensitive (Bullrich et al. 2002; Gawroński et al. 2014). This means there is a possibility of manipulating *Eps* genes to increase yield and optimise adaptation. Grain quality can also be improved by manipulating *Eps* loci given that Herndl et al. (2008) showed that *Eps* together with the major genes that control vernalization and photoperiod flowering influence grain protein content.

However, presently there is scant information on the identity of *Eps* genes, and the mechanism of control that these *Eps* genes employ in hexaploid wheat. For instance, it is not certain whether *Eps* genes act independently of environmental cues (Cockram et al. 2007; Laurie et al. 2004; Bullrich et al. 2002), although many reports suggest that this is the case (Lewis et al. 2008; Cockram et al. 2007; Bullrich et al. 2002). Appendino and Slafer (2003) showed that *Eps* genes could respond to temperature. Laurie et al. (2004) underscored the need to study more about *Eps* genes given that little is known about them despite their immense potential in improving plant breeding. This was alluded to by Cockram et al. (2007) who suggested that *Eps* genes were a potential source of variation in targeted breeding given that they were present in both winter and spring crops. The *Hordeum vulgare EPS2* locus on chromosome 2H (Laurie et al. 1995) was also reported to be orthologous with the wheat group 2 loci (Laurie 1997). The candidate gene for this locus has only been recently shown in barley to be a homolog of the *Antirrrhunum* gene *CENRORADIALIS* (*CEN*) designated *HvCEN* (Comadran et al. 2012). Mutations at this gene were shown to cause the wild type indeterminate inflorescence of *Antirrhunum* to terminate into a flower (Bradley et al. 1996). Analysis of the *HvCEN* alleles led to the conclusion that *HvCEN* was important for geographic range extension as well as influencing the gradual separation between spring and winter barley (Comadran et al. 2012). The orthologue of this gene is yet to be identified in the economically important hexaploid wheat.

Following the work done by Griffiths et al. (2009), Zikhali et al. (2014) reported the validation of an *Eps* effect on 1DL in hexaploid wheat (Fig. 39.2). Near isogenic lines (NILs) of a cross between wheat varieties Spark and Rialto grown in the field and controlled environments enabled the QTL on 1DL to be defined as an *Eps* effect (Zikhali et al. 2014). The NILs segregated for heading date both in short and long days (Fig. 39.2) when fully vernalized (Zikhali et al. 2014). Zikhali et al. (2014) reported that *Triticum aestivum FLOWERING LOCUS T 3* (*TaFT3*) was not a candidate for the 1DL *Eps* effect. The 1DL *Eps* locus was reported to be most likely an orthologue of *Eps-Am1* and the genes *MOT1* and *FtsH4* were suggested as possible candidates for 1DL. In addition to *MOT1* and *FtsH4*, the gene *T. aestivum EARLY FLOWERING 3* (*TaELF3*), a circadian clock gene, was also suggested as a possible candidate for 1DL given that it also falls in the QTL interval of 1DL (Zikhali et al. 2014).

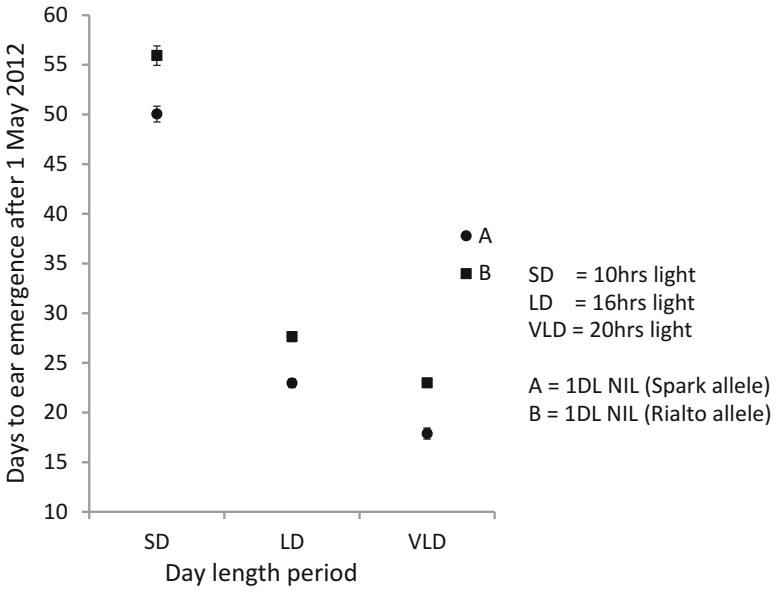

Fig. 39.2 Zadoks growth stage 55 for leading tillers of Spark X Rialto NILs grown under controlled environments. The heading days are the mean of 24 plants for the Spark (A) NIL and 30 plants for the Rialto (B) NIL. The additive effect is about five days in the three photoperiod treatments. Student's *t*-test was carried out for the mean heading days and all the four NILs pairs have a p value <0.0001, which is highly significant. The error bars are the Standard error of the mean (Adapted from Zikhali et al. (2014))

In a nutshell, *Eps* genes are gradually being understood with some QTL loci already cloned like the *Eps-3Am* locus in *T. monococum.* It is also becoming apparent that *Eps* genes may not be independent of environmentally cues as previously understood. For example the *Eps-3Am* locus has been found to have a circadian clock effect, which suggests that this gene responds to photoperiodic changes (Gawroński et al. 2014). Again the thermo sensitivity of both the *Eps-Am1* and *Eps-3A* loci (Gawroński et al. 2014; Bullrich et al. 2002) further suggests that *Eps* genes are not independent of environmental cues. A more accommodating definition of *Eps* would be the variation that is observed in flowering time when both vernalization and photoperiod requirements are fully met without being necessarily independent of environmental cues. The additive effect from multiple *Eps* loci maybe important for wheat adaptation and fine-tuning flowering time.

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