QTL mapping for early ground cover in wheat (*Triticum aestivum* L.) under drought stress

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Early vigour had been a target trait for developing wheat varieties tolerant to moisture stress. Manifestation of this trait depends on the relative efficiency of a genotype to utilize the residual soil moisture and dew precipitation, thereby developing a good canopy in lesser time after emergence. Lack of proper quantification system had always prevented the use of early vigour as a dependable selection parameter under field conditions. Digital imaging intervention has facilitated phenotyping this parameter in the form of early ground cover (EGC). Utilizing this phenotyping strategy, we have identified a quantitative trait locus for EGC located on the chromosome 6A (short arm) with a significant additive component under moisture stress in the north western plain zone of India.

Keywords: Digital ground cover, drought tolerance, early ground cover, quantitative trait loci, wheat.

OVER 68–70% of arable area in India is vulnerable to drought during cropping seasons[1]. Wheat producing belts of the Indian subcontinent are dependent on the residual soil moisture of monsoon. Irregularities in monsoon and erratic rainfall patterns in recent times have further jeopardized *rabi* farming. For wheat varieties developed for the tracts lacking irrigation, early vigour trait is a desirable parameter which promotes tillering (productive tillers) under residual soil moisture early ground cover (EGC), which is a measurable manifestation of early vigour, determines the initial crop stand by building up the canopy foliage. There are various characteristics that account for early vigour namely length of coleoptile and coleorhiza, rate of germination, phyllochronical attributes, etc. for whom phenotyping under field conditions is tedious. EGC scored at regular time intervals after emergence has been found to be a good indicator for expression of early vigour in terms of vegetative growth, resulting in higher photosynthate accumulation. A canopy with optimal biomass gives the genotype a better intercept of incident solar radiation, thereby decreasing evaporation from soil surface[2]. EGC increases water use efficiency and empowers the crop for competitiveness with weeds and potentially also decreases soil erosion. The relation between EGC or digital ground cover (DGC) with soil water holding capacity of the surface soil of the root zone has been well studied and documented[2,3].

Conventionally, early vigour was measured by visual indices in an ordinal scale. Abstract phenotyping has prevented utilization of optimal early ground cover as a distinct stress avoidance/tolerance parameter till the advent of digital image analysis interventions under field conditions. Mullan and Reynolds[3] introduced a simplified digital image analysis approach for precise quantification of the EGC trait. Since then, digital pixel analysis has evolved to be a considerable high-throughput phenotyping method for drought escape and avoidance. To identify informative genomic region(s) housing the QTL(s) and linked makers for EGC in wheat, the present study was undertaken for utilization in variety development.

Recombinant inbred line (RIL) population generated from the cross of HD2877 × HW2004 was taken for QTL mapping for EGC in the *rabi* season of 2011–12. It comprised three hundred RILs in $F_{9:10}$ generation. The donor parent, HW2004 is a near isogenic line developed from the national check variety for drought and heat tolerance C306. The parentage of HW2004 is C306*7//TR380-14(Lr24), i.e. C306 background carrying the gene for leaf rust resistance *Lr24*. The parentage of HD2877 is CDWR9549/HD2347//HD2402. The parental lines were studied for EGC along with the national check for two seasons (*rabi* 2010–11 and 2011–12) in replicated trials under irrigated and rainfed conditions to identify the extent of contrast. The response of EGC to different water regimes was studied by paired *t*-test of the means of the parents. Analysis of contrast between the donor and recipient parent under a particular moisture regime was carried out by Student's *t*-test.

The trial was conducted in augmented design[4] in the experimental farm at Indian Agricultural Research Institute, New Delhi (28°38′N and 77°12′E, 293 m elevation from sea level). It comes under the north western plain zone (NWPZ) of wheat production having a subtropical semi-arid climate; clay loam textured alluvial soil (Typic Haplustept), slightly alkaline in pH and low organic matter content. The RILs were sown in two rows of 4 m length, with row-to-row distance of 40 cm and plant-to-plant distance 30 cm under rainfed condition. Standard agronomic practices followed to grow the crop which

Table 1. Analysis of parental contrast, EGC means of the genotypes under different water regimes

Genotypes	2010–11			2011–12		
	Irrigated	Rainfed	Paired t (P)	Irrigated	Rainfed	Paired t (P)
HD2877	170.68	166.2	0.714	184.15	177.81	0.689
HW2004	178.42	126.37	0.156*	188.43	155.4	0.268*
Student's t-test (P value)	0.658	0.192*	–	0.734	0.16*	–
C306	198.37	178.23	0.289*	190.52	181.7	0.342*

*Symbolizes probability (P value) of the population means being contrasting at 5% level of significance.

thrived on residual soil moisture and dew precipitation. The parental lines and RILs were phenotyped as per the DGC analysis method of Mullan and Reynolds[3]. Phenotyping was carried out at 4-day interval after emergence till Zadok's stage number thirty five (Z.35, fifth node visible in the central culm)[5], and the stage of contrast between the parental lines was identified for phenotyping the RILs. For capturing the images, digital camera (Fujifilm, model: S series, Finepix SL1000) was used and the consequent pixel analyses were carried out in the portable version freeware of Adobe CS3 software. The phenotypical data was statistically analysed in the R programming environment software[6]. Descriptive statistical parameters from the population data were analysed in the 'pastecs' package of R. Euclidian clustering was done using the 'cluster' and 'ape' packages of R. Variance components of the field trial in the augmented design were analysed in the 'agricolae' package of R.

The RIL parental lines HW2004 and HD2877 were screened for polymorphism in genetic background by a panel of seven hundred simple sequence repeat (SSR) markers pooled from Somers et al.[7]. DNA extraction protocol modified from Prabhu et al.[8] was followed. For SSR genotyping of the parental lines and RILs, standardized thermal cycling protocol from Singh et al.[9] was adopted. Out of the polymorphism survey, 92 polymorphic SSRs spanning the whole genome were identified and a linkge map was prepared by using MAPMAKER program (Macintosh V2.0)[10]. The consequent QTL mapping was done in the Windows version of QTL Cartographer V2.0 (ref. 11).

EGC trait per se imparts drought avoidance in the genotype by enabling it to thrive on residual moisture and attain optimal vegetative growth by utilizing the dew precipitation to confront the post-anthesis drought and heat stress. The stage of expression of parental contrast for EGC under different water regimes was defined in terms of Zadoks scale[5]. The behaviour of the trait in population was duly studied and a single QTL linked to the trait is hereby reported.

The parental lines HW2004 and HD2877 along with the national check C306 were grown in replicated trials under rainfed and irrigated conditions during rabi 2010–11 and 2011–12 and expression of contrast was recorded (Table 1). The mean surface soil field capacity (θ_{FC}%

weight/weight at surface to 5 cm depth) varied from 21.3 during morning hours (7–8 : 00 am, under dew precipitation) to 18.6 (1 : 00 pm afternoon, under bright sunshine). The average bright sun shine hours in the first fortnight of February ranged from 4 to 6 h for both the seasons (rabi 2010–11 and 2011–12) when the crop was at tillering. The crop canopies were progressively analysed for EGC from tillering initiation to complete tillering (Z.21–29), when distinct parental contrast was apparent. The parental contrast data are displayed in Table 1. The parental lines exhibited highest contrast at the stage Z.23–24 (central culm with 2–3 tillers) under rain-fed conditions of the north western plain zone. In Figure 1, the contrast expression of HD2877 and HW2004 with respect to EGC is shown along with C306 under rain-fed condition. Before and after this stage the magnitude of contrast reduced. Under irrigated condition, the parental lines HW2004 and HD2877 exhibited negligible contrast in growth, all along the tillering stage. Paired t-test confirmed significant difference in the EGC of C306 and HW2004 under rain-fed and irrigated conditions, whereas HD2877 exhibited marginal difference over the cropping seasons. Thus it can be rationalized that C306 and HW2004 respond more to irrigation with respect to HD2877 and manifest it in the form of higher EGC. Student's t-test confirmed significant contrast between parental entries (HD2877 and HW2004) under rain-fed conditions and marginal contrast under irrigation at Z.23-24. It was apparent from the behavioural elasticity of HW2004 with respect to the ground cover under irrigated and rain-fed conditions, in both the cropping seasons, there may be some subtle genetic factor or genotype × environment interaction linked to such a type of expression. The variation may also be accounted for the temporal tillering pattern of the concerned variety, i.e. whether the genotype is rapidly tillering consecutively or develops a new tiller only after attaining a certain growth in the existing ones. Different such genetic models of tillering behaviour of spring wheat had already been reported[12]. Vegetative growth patterns of those RILs may be studied under varied conditions in future.

The adjusted plot means of $F_{9:10}$ RILs varied from 41.74 to 188 pixel units in terms of their EGC during rabi 2011–12 under NWPZ of Indian subcontinent. With respect to the EGC scores of parental lines (Table 1),

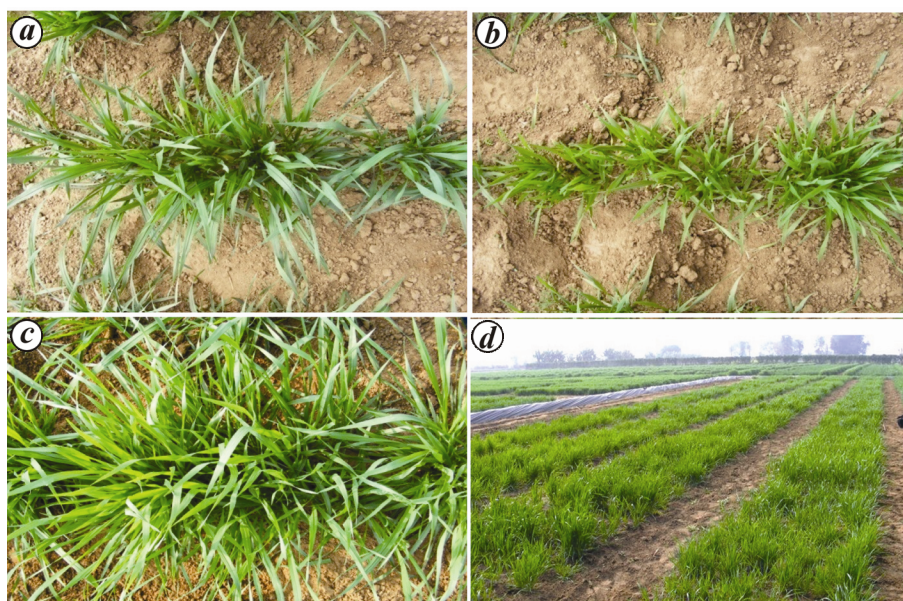**Figure 1.** Early ground cover/digital ground cover phenotypes of the parental lines: *a*, HD2877 (*Z.23–24*); *b*, HW2004 (*Z.23–24*); *c*, Indian national check for drought tolerance C306 (*Z.23–25*); *d*, View of the field trial (RILs) under rainfed conditions at Zadok's 23–24 stages.
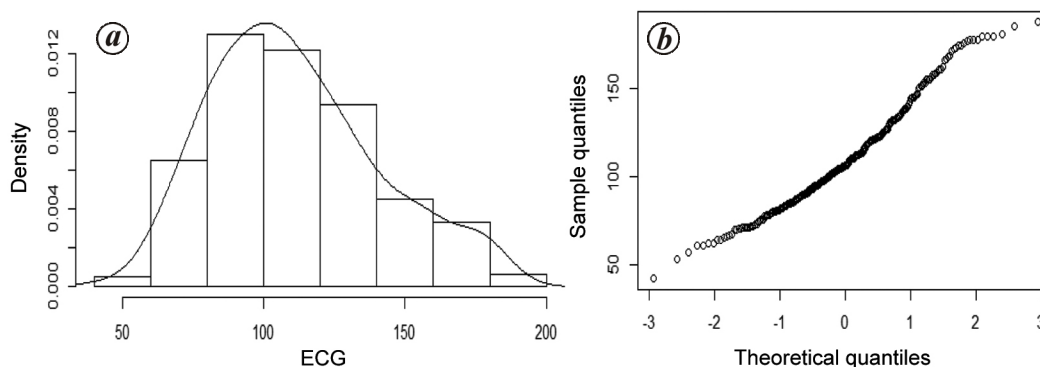

Figure 2. *a*, Population distribution of the RILs for EGC; *b*, Quartile–quartile plot of the distribution.

this variation was significant. The population had a general mean of 111.12 pixel units concerned to EGC with a standard error of 1.73 and a confidence interval of 3.40 at 5% level of significance. The standard deviation from the population mean was 29.95, with a coefficient of variation of 0.27. The treatment (RILs) residuals exhibited a diffused random plot (not shown) predicting near homogeneity of the experimental plot. The descriptive parameters indicated a normal distribution of EGC (Figure 2 *a*) in the RILs which was further subjected to the Shapiro-Wilk's test of normality and quartile-quartile plotting. The Shapiro-Wilk's test of normality found W value = 0.97 ($P = 1.131e^{-05}$) for the trait in the concerned RILs and confirmed the normality of the distribution, while the quartile-quartile plotting also generated a nearly linear plot confirming the same (Figure 2 *b*). The population distribution of EGC implied the underlying quantitative genetic control of the trait. In the population of 300, 35

RILs were above the mid-parental value and 20 RILs superseded the superior parental mean for EGC. Two hundred and sixty four RILs under-performed with respect to the EGC mean of the contrasting parent HW2004. It is apparent from such results that interaction effects were responsible for the expression of EGC under field conditions[3].

The factors imparting variance in the population for EGC laid out in augmented block design were duly analysed under unidirectional elimination of heterogeneity separately for blocks and treatments (RILs) respectively (Table 2). The critical differences (CD) per se briefed the response of the RILs for EGC (Table 3). The CD for controls confirms the uniform contrast expression across the blocks. Between the blocks CD being higher with respect to within the blocks, implicitly inferred that environmental heterogeneity was insignificant within the blocks. Equivalent CD for the treatments within block and

between control and augmented treatment also infer lesser microenvironment effect within the blocks. Under block adjustment, variance due to augmentation, control versus augmentation and block per se were found to be significant. It implies that considerable environmental interaction effect contributes to the overall variation. Under heterogeneity elimination for the treatments (RILs), control + control versus augmentation and treatments per se showed significant variance. The controls were found to be varying under heterogeneity elimination (adjustment) for blocks and treatments separately. It indicated uniform expression of contrast and high environmental interaction for the expression of EGC, but their mean sum of squares (MSS) was equivalent under both cases. It implied that some fixed effect was making the controls to vary. The MSS of the treatments or RILs was found to be equivalent both under adjustment of blocks and treatment per se, which was an intriguing result. Significant variation in the population for EGC due to genotypic effect (treatment or RILs) under both the cases of heterogeneity elimination and equivalent MSS of the treatments indicated towards the sound genetic component of the trait (Table 2). The genotypic variance for EGC was found to be ranging from 864.2 (treatment adjusted) to 979.8 (treatment unadjusted) with a minor residual MSS of 17.1, thereby phenotypic variance ranging from 881.3 to 996.9. In contrast to this result, under both the cases of heterogeneity elimination, MSS due to blocking effect were significantly different. Lower value of the residual MSS under both systems of heterogeneity elimination confirmed the precision of the experimental set-up and results.

Being the stress-tolerant counterpart, HW2004 underperformed to HD2877 (agronomically superior line with respect to yield) concerned to the early ground cover under moisture stress for both seasons. However, C306, the donor line of HW2004, performed high for early ground cover trait per se under moisture stress during *rabi* 2010–11 and 2011–12 in comparison to HD2877. From this result, it can be conceptualized that the genetic background of HW2004 suppressed the expression of early ground cover trait, which expressed predominantly in its donor line C306 under moisture stress.

Mapping for EGC in $F_{9:10}$ RILs in the *rabi* 2011–12 identified the quantitative trait loci '*Qtld.egc*' was identified in the short arm of the chromosome 6A with a LOD value of 3.14 (Figure 3). QTL had high additive component ($A = 7.2803$) and contributed by the donor parent HW2004 for drought tolerance or avoidance, in spite of lower performance compared to EGC with respect to HD2877. Though HW2004 under-performed HD2877 compares to the expression of EGC under moisture stress, it carried all the genetic components for responsible for trait expression in its genetic background. It can be conceptualized that higher performance of HD2877 may be due to its superior agronomic background which utilizes the environmental factors better in expression. However

Qtld.egc had zero dominance component of variance. QTL spanned around the SSR interval from 18.31 cM (Xwmc 553) to 36 cM (Xgwm 427). This marker interval was close to the centromeric region of the chromosome[7]. *Qtld.egc* had a recombination frequency of 0.001 with the left flanking SSR Xwmc 553 and 0.1699 with the right flanking Xgwm 427, the result validated its near-centromeric location where crossover frequency is generally less[7,13]. The QTL detection window generated by the QTL cartographer[11] on the basis of marker-trait linkage, also showed SSR Xcfd80 linked to Qtld.egc. Marker Xcfd80 being located putatively at near-telomeric region (Figure 3) may serve as a linked marker for this trait and may be useful for future QTL studies for EGC pertaining to chromosome 6A, where SSR resources are comparatively limited. Low recombination frequencies[13] of the flanking SSRs indicate expected repeatability of the QTL identified under NWPZ condition and their use in preliminary marker assisted selection programmes for similar climatic conditions across the globe. Proportion of the variance explained by QTL conditioned on the background SSRs and residual variables was 4.37% which suggest *Qtld.egc* to be a minor QTL[14]. However, solitarily along with background SSRs *per se* and residual variables, *Qtld.egc* explained 11.7% of the total phenotypic variance. Eventually >10% explanation of the total

Table 2. Analysis of variance of augmented design for EGC in the RILs ($n = 316$)

Factors of variance (treatment adjusted)	MSS	P (>F value)
Block unadjusted	5140.3	
Treatment adjusted	881.3	6.786e-06***
Control	1044.1	0.0001051***
Control + control versus augmentation (block adjusted)	880.7	6.801e-06***
Treatment unadjusted	996.9	
Block adjusted	170.2	0.0035361**
Control	1044.1	0.0001051***
Augmented	896.9	6.389e-06***
Control versus augmented	30843.9	1.038e-09***
Residuals (both cases)	17.1	

The mean sum of squares (MSS) and P values of respective factors imparting variance separately under block and treatment adjustments are provided. Probability of being variant (P values) is expressed with respect to F-values and the following codes for indicating level of significance used – 0***, 0.001 **, 0.01 *, 0.05, 0.1 ' ' and 1.

Table 3. Threshold CD values for pair-wise comparison of RILs under different conditions.

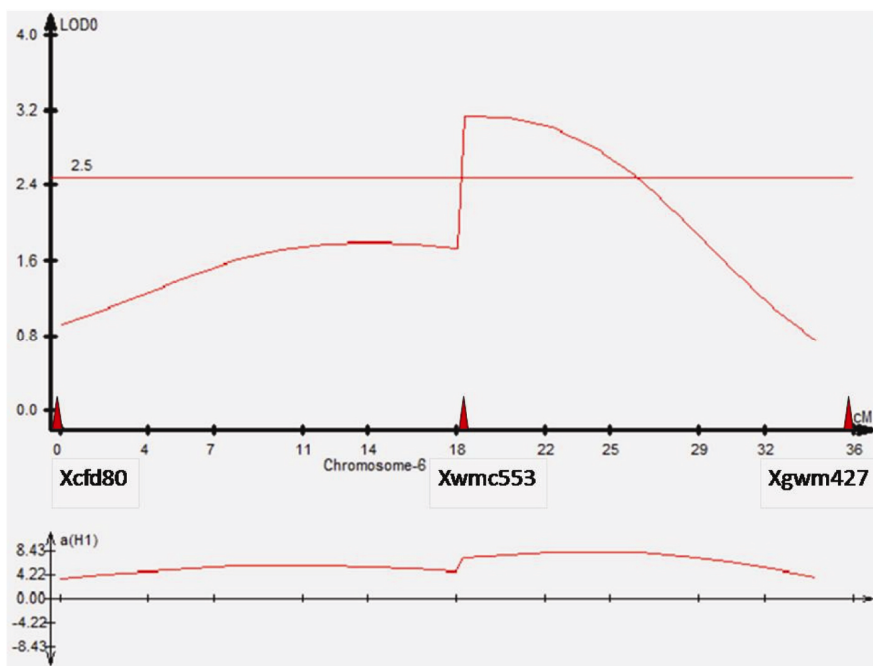| Critical differences (between)	Standard error difference
Two control treatments	2.065060
Two augmented treatments (same block)	5.840873
Two augmented treatments (different blocks)	7.153579
An augmented treatment and control treatment	5.162651

Figure 3. QTL detection window of 'Qtld.egc' on chromosome 6A with a LOD value of 3.14 and an additive component (A) value of 7.2803 identified for the expression of early ground cover in spring wheat.
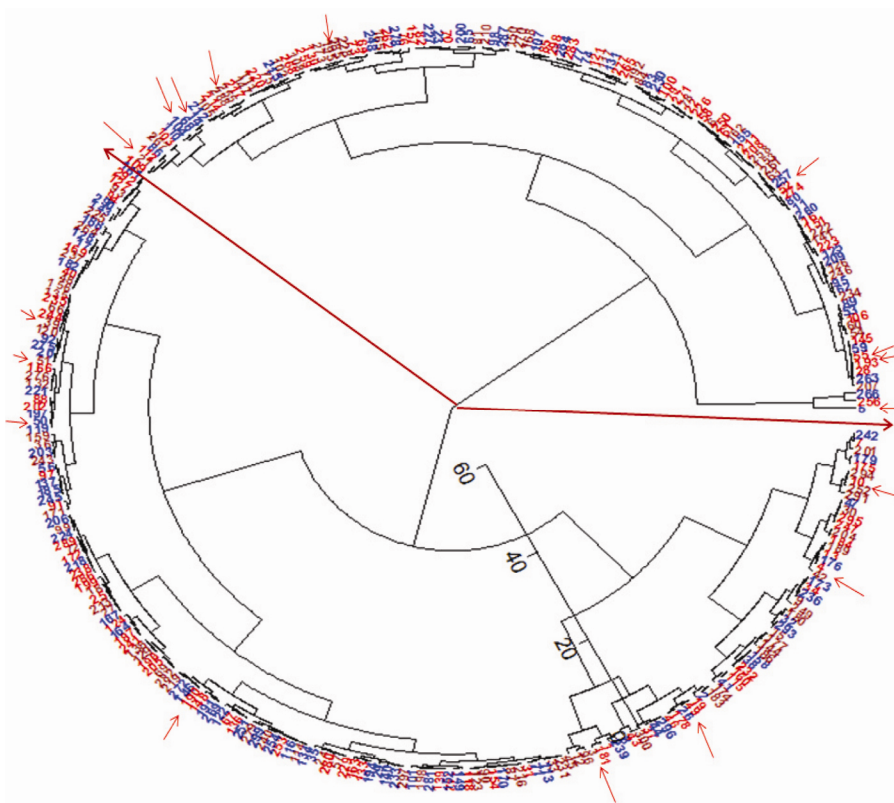

Figure 4. Clustering of the RILs based on Euclidian dissimilarity coefficient for EGC under drought, transgressive segregants are indicated by arrow marks.

phenotypic variance under certain conditions designates it as a major QTL as well[14]. Thus Qtld.egc has all the potential to act as major QTL under certain environmental conditions which need to be further identified

through multilocation trials. The chromosome 6A of wheat genome had figured many times in the reports regarding QTL mapping for drought or heat tolerance in the past. Pinto et al.[15] reported that QTLs (up to 2010) for

several yield and physiological parameters under drought and heat are located on chromosome 6A, viz. yield, grain number/m^{-2}, 1000 grain weight, grain size, grain filling efficiency, plant height, canopy temperature and chlorophyll content. The authors also reported QTLs for normalized difference vegetation index (NDVI) collocating in the same chromosome. Location of the NDVI QTLs at vegetative and grain filling stages on 6A[15] is quite significant result in terms of the present report, because this trait is essentially a function of the ground cover developed by the plant canopy. NDVI is phenotyped by green seeker sensors which are sophisticated and high end equipment. Mullan and Reynolds[3] reported high correlation between digital ground cover and NDVI. The present study is an indication that such low input phenotyping method can replace green seeker sensor. Obviously further studies are needed on field phenotyping for validation. Along with the above mentioned traits, QTLs for parameters relating to the spectral photochemistry relating to the photosystem II had also been reported in 6A by Yang et al.[16] and Kumar et al.[17].

Figure 4 shows the cluster dendrogram of the RILs based on Euclid dissimilarity coefficient. The RILs formed 2 distinct clusters (separated by radial lines originating from the centre of the dendrogram). The expected clustering in an advance generation RIL population is approximately 1 : 1 (AA : aa) due to fixing of the alleles, here also two distinct clusters were observed. The RILs performing superior to the better parent (HD2877) with respect to EGC are earmarked by arrow marks in Figure 4, presented a diffuse pattern in the clustering. This may be due to the fact that both the specific trait linked genic region per se of HW2004 and superior background of HD2877 equivalently contribute to higher EGC expression in the advance generation RILs at the Delhi location. Out of the transgressive segregants, some of them were performing due to the trait-linked genic region of HW2004 or superior background effect of HD2877 or interaction of both along with environmental effect. Further multilocation trials of such elite segregants will reveal their genetic worth regarding EGC.

Thus a genomic location accountable for early ground cover trait was identified in the short arm of chromosome 6A in Indian spring wheat. EGC being strongly related with drought responsive physiological parameters like NDVI, leaf area index, spectral interception of the canopy[2] its response in correlated selections need to be tested using conventional and marker-assisted interventions. The detected QTL provides a genetic basis to the EGC trait for use in physiological breeding for drought tolerance under field conditions in the subcontinent.

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