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Quantitative Trait Loci Associated to Drought Adaptation in Pea (*Pisum sativum* L.)

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Abstract Pea (*Pisum sativum* L.) is an important grain legume whose yield stability and production is constrained by drought stress in most environments. However, little is known on availability of drought adaptation sources and its genetic control in pea. The aim of this work was to study the genetic of drought adaptation in pea and identify the genomic regions controlling the trait. Towards this objective, in this work, we assessed drought symptoms and relative water content in soil (RWCS) and leaves (RWCL) along a time course of water stress on a pea Recombinant Inbreed Lines (RILs) population from two parents known to segregate for drought adaptation. Drought adaptation in this population was a quantitative trait. QTL analysis using composite interval mapping (CIM) and multiple interval mapping (MIM) allowed us to identify ten quantitative trait loci (QTLs) associated with the traits explaining individually from 9 to 33 % of the phenotypic variation depending on the variable assessed and altogether from 20 to 57 %. A set of reproducible markers linked to these QTLs (A6, AA175, AC74, AD57, AB141, AB64, *Psblox2*, PsAAP2_SNP4, and DipeptIV_SNP1) were identified. These markers can be used to select the individuals harbouring the desired QTLs in pea breeding programs for drought adaptation.

Keywords Drought · Relative water content · Pea · QTL

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Introduction

Pea (*Pisum sativum* L.) is one of the most widely grown grain legumes in the world with primary production in temperate regions (Smýkal et al. 2012). One of its advantages as a legume crop relies on its capacity for symbiotic nitrogen fixation, allowing the reduction in the use of fertilisers in crop rotations. However, this process is highly sensitive to abiotic stresses such as drought and salinity (Zahran 1999) which are major constraints to the production and yield stability of pea, especially during the flowering and pod filling (Doré et al. 1998).

Water deficit induces a range of physiological and biochemical responses within the plant which include stomatal closure, activation of respiration, repression of cell growth and photosynthesis. At the cellular level, plant responses to water deficit may result from cell damage, whereas other responses may correspond to adaptive processes (Araújo et al. 2015). Dehydration in plant tissues induces changes in cell membrane stability and permeability, which finally lead to changes in the cell functions. Therefore, different mechanisms of response and physio-biochemical changes at both cellular and whole plant level are induced by drought, making of it a complex abiotic stress which tolerance is supposed to be controlled by different genomic regions (Shinozaki and Yamaguchi-Shinozaki 2007; Xoconostle-Cázares et al. 2011).

Visual assessment of drought symptom evolution along a time course has been described as a fast approach to discriminate between tolerant and susceptible plants (Iglesias-García et al. 2012; Sánchez-Martín et al. 2012). The development of visual scales specifically adapted to a crop allows to indirectly assessing a wide range of factors, not only the ability of the plants for water uptake and its preservation but also others such as oxidative stress (by photoinhibition, lipid peroxidation, etc.) which could be yellowing the leaves.

As a complex trait, drought assessment would improve from its partitioning into components that are easy to measure such as relative water content (RWC) in soil (RWCS) and leaves (RWCL). Whereas RWCS has not been associated to adaptation or susceptibility to drought, it is a helpful parameter which allows indirect assessment of the water uptake by the plants. If RWCS decreases quickly, it could reflect a higher stress level within the plant than indicated by a slower decrease, but not necessarily lower plant efficiency under water stress conditions. Water use efficiency (WUE) by the plant is then strongly related with RWCS when adaptation and susceptibility to drought is assessed, as if a plant shows a high water uptake but a low WUE, it will have to deal with both these circumstances to survive during drought periods.

On the other hand, relative water content in leaves (RWCL) reflects the ability to maintain cell turgor when measured under drought stress, providing an idea of the adaptation capacity of the plant. Thereby, RWCL has been widely used as a physiological index in different crops for the evaluation of drought and temperature adaptation (Hunt et al. 1987; Tripathy et al. 2000; Siddique et al. 2000). The identification of the genes controlling RWC as well as molecular markers tightly linked to them by QTL mapping could be used as a tool to assist breeding for water stress adaptation. However, despite being a suitable index for water stress, the genes controlling RWC still remain unknown. No previous QTL analysis for drought adaptation have included this trait, probably because the measurement of this trait in large segregating populations can be tedious and expensive (Keurentjes et al. 2008).

The main objective of this work was to study the genetics of drought adaptation in pea and to identify molecular markers which can be useful to select tolerant genotypes in breeding programs. With this aim, we assessed the evolution of drought symptoms and RWC in soil and leaves along a water stress time course in a recombinant inbred line (RIL) population segregating for drought adaptation and performed a QTL analysis.

Materials and Methods

Plant Material and Growth Conditions

The population used in the study consisted of 98 $F_{7:8}$ RIL families along with their parental lines P665 and cv. Messire. P665 (derived from the ICARDA accession IFPI3280) is a *Pisum sativum* subsp. *syriacum* full-leafed accession widely used in pea breeding due to its resistance to diseases (Fondevilla et al. 2005, 2010; Rubiales et al. 2009a, b) in spite of showing wild traits such as late flowering, creeping growth habit and violet flowers. Messire is a *P. sativum* subsp. *sativum* full-leafed, early-flowering and white-flowered pea cultivar. The parents P665 and cv. Messire have been previously characterised as tolerant and moderately susceptible to water stress, respectively, according to a visual scale and other drought-related traits (Iglesias-García et al. 2012). Thus, P665 maintained the highest carbon fixation rate under drought conditions whereas Messire showed a strong decrease on conductance under water stress (Iglesias-García et al. 2012).

Seeds from all the RIL families along with the parents were pre-germinated in Petri dishes with moistened filter papers in the dark for 48 h in a cold chamber at 4 °C and then placed for another 48 h in a growth chamber at 65 % relative humidity and 20 °C during all the 12-h day/12-h night photoperiod. Seedlings were planted individually in 0.5-L pots filled with peat/sand (3:1) and placed into a growth chamber in a randomised complete block design with nine replicates. Plants were grown at 21 °C, under a photons flux density (PPFD) of 200 μmol m^{-2} s^{-1} supplied by high-output white fluorescent tubes during all the 12-h day/12-h night photoperiod.

Drought Stress Treatment

Drought experiments were carried out at seedling stage (3–week-old plants) (Xiao et al. 2007; Hao et al. 2009; Gong et al. 2010; Sánchez-Martín et al. 2012). During growth, trays carrying the pots were watered regularly. At day 21, water was withheld from those plants selected for drought treatment (Hao et al. 2009; Gong et al. 2010) for a period of 15 days when the mean RWCS reached approximately 23 % for the RILs and their parents. Control plants were watered as described above throughout the whole experiment.

Since several of the evaluations performed are destructive (i.e., RWCL), nine plants (including an extra plant in case of any plant damage during the experiment) per RIL or parent and treatment were grown. The no-destructive evaluations such as the visual scale of the soil RWC assessment were performed on the complete set of plants, whereas the destructive evaluation was performed on four replications per sampling time, each replication consisting of a leaflet of the fourth pair.

Visual Scale Assessment of Water Stress Symptoms

From the beginning of the watering withdrawal, all the nine replicates of each RILs and their parents were assessed daily with a visual scale adapted to pea behaviour from the one developed for oat (*Avena sativa*) by Sánchez-Martín et al. (2012). According to previous experiments (Iglesias-García et al. 2012), we used the fourth pair of leaves to evaluate drought symptoms uniformly on each line.

We assessed temporal evolution of water stress symptoms according to five stages, numbered from 1 to 4 (Online resource 1). Each status in the scale corresponds with the following characteristics: (1) no symptoms observed in the pair of leaves; (2) general softening of the pair of leaves; (3) curved leaves with marked ribs; (4) yellowing and/or border necrosis observed (0–50 % of the pair of leaves).

Finally, data obtained were used to calculate the area under the drought symptoms progress curve (AUDPC) for each genotype, according to the following equation: $AUDPC\_VS = \Sigma[(x_i + x_{i+1}) / 2] \times (t_{i+1} - t_i)$

where x_i = water stress symptoms at date i, x_{i+1} = of water stress symptoms at date $i + 1$, and $t_{i+1} - t_i$ = number of days between scoring dates i and $i + 1$, which was one in all cases as it was the interval between the assessed time points. This trait was named "AUDPC_VS."

Relative Water Content Measurements

RWCS was calculated daily according to the methodology used by Bechtold et al. (2010). Briefly, pots for all the RILs together with the parental plants were filled with identical amount of substrate. Three additional pots were used to determine the weight of pots after freely water and let drainage for 2 h (saturated soil, SW) or dried for 5 days at 60 °C when they reached an stable weight (dry soil, DW). Fresh pot weight (FW) was determined daily for all the RILs together with the parental lines and RWCS was calculated according to the formula RWCS = (FW − DW)/(SW − DW). RILs and parental lines were left to dry until a mean of approximately 23 % RWCS was reached.

RWCL was determined according to Cabrera-Bosquet et al. (2007). One leaflet of the third pair of leaves of each plant was collected for this measurement in accordance with RWCS assessment. Thus, samples were taken 0, 4, 9 and 15 days after watering withdrawal, corresponding to 100, 75, 45 and less than 23 % of average RWCS, respectively. Six hours after the onset of the light period, leaf blade segments were weighed (fresh weight (FW)), floated on distilled water at 4 °C overnight and weighed again (turgid weight (TW)). They were then dried at 80 °C for 48 h. After this, the dry weight (DW) was determined. RWC was then calculated as RWC = (FW − DW) (TW − DW) − 1 × 100. As both RWCS and RWCL were taken along a time course, we calculated the area under the drought progression curve (AUDPC_RWCS and AUDPC_RWCL, respectively) using the following formula: $AUDPC\_RWCS/L = \Sigma[(x_i + x_{i+1}) / 2] \times (t_{i+1} - t_i)$.

where x_i = estimated proportion of water content at date i, x_{i+1} = estimated proportion of water content at date $i + 1$, and $t_{i+1} - t_i$ = number of days between scoring dates i and $i + 1$. This trait represented the evolution of RWCS (AUDPC_RWCS) and RWCL (AUDPC_RWCL) along the time course for each RIL and the parents.

Statistical Analysis and Heritability Estimation

Statistical analyses were performed using SAS ver. 9.2 (SAS Institute Inc. 2004). Standard analysis of variance (ANOVA) was performed using PROC GLM to determine variation in AUDPC_VS, AUDPC_RWCS and AUDPC_RWCL, $RWCS_F$ and $RWCL_F$ (ANOVA results and descriptive statistics are provided in Online resources 2 and 3, respectively). The trait named $RWCS_F$ represents the value of RWCS 15 days after watering withdrawal (which was the total extension of the drought period), whereas the trait $RWCL_F$ refers to the value of RWCL for each RIL and their parents when the respective RWCS reached less than 23 %. Variance components were estimated using PROC VARCOMP.

Broad sense heritability (h^2) that represents the part of genetic variance in the total phenotypic variance was calculated using the following formula: $h^2 = \delta^2_g / (\delta^2_g + \delta^2 / r)$, where δ^2_g is the genotypic variance, δ^2 is the error variance and r is the number of replications. Normality of residual distribution was checked using the Kolmogorov–Smirnov test. Pearson correlation coefficient between traits was estimated using PROC CORR in SAS.

QTL Analysis

QTL analysis was conducted over a previous genetic map developed using the RIL population of the cross P665 × cv. Messire (Carrillo et al. 2014) using composite interval mapping (CIM) and multiple interval mapping (MIM) in Windows QTL Cartographer V2.5 (Wang et al. 2011). Markers to be used as cofactors for CIM were selected by forward–backward stepwise regression. The number of markers controlling the genetic background in CIM was set to five. The thresholds for the detection of QTLs were estimated by permutations analysis (Churchill and Doerge 1994) using 1000 permutations. One- and two-LOD support intervals for the position of each QTL were calculated as described by Darvasi et al. (1997).

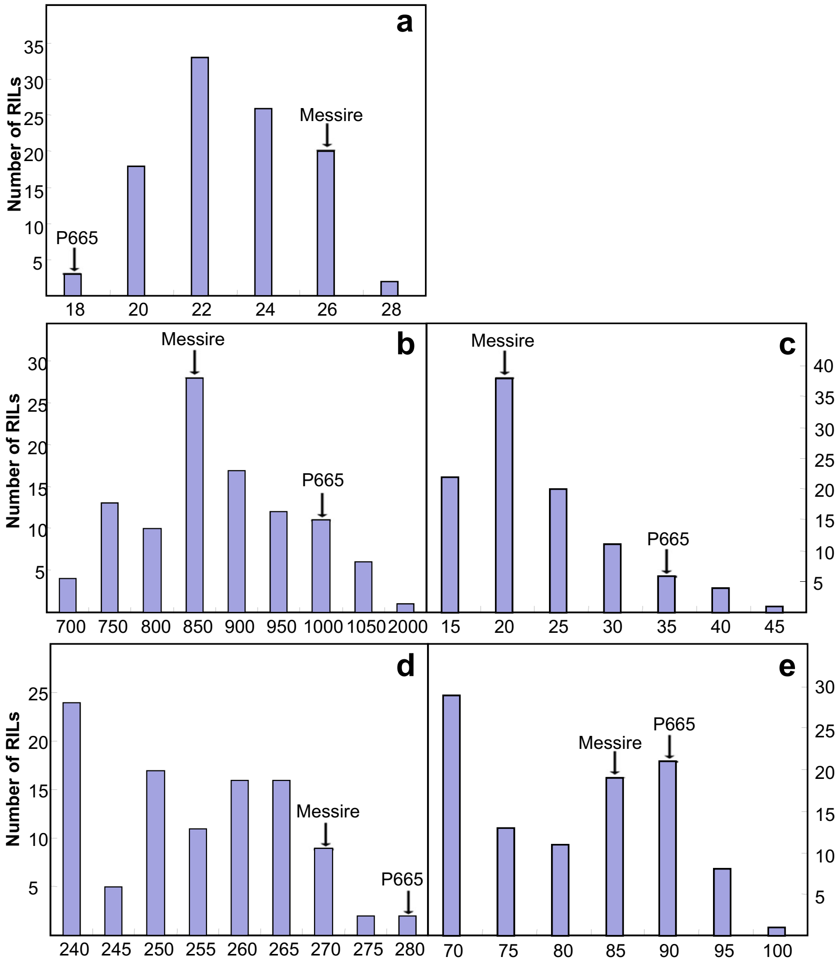
To obtain more precise information on QTL effects and positions and to evaluate for the presence of digenic epistatic interactions across the QTL pairwise combinations, MIM (Kao et al. 1999; Zeng et al. 1999), as implemented in WinQTL Cartographer, was used by considering as initial QTL models the CIM results obtained for the trait. The initial CIM-derived QTL model was subjected to a search for significant epistatic interactions among QTLs. Both main additive effects and their epistatic interactions were tested for significance using the Bayesian information criterion (BIC) with the penalty function $c(n) = \log(n)$, with n (sample size) = 98 (Zeng et al. 1999). The final main additive and epistatic QTL effects and the R^2 values of the model were then estimated.

Results

Assessment of Drought-Related Parameters

The parent P665 showed very low drought symptoms with a mean AUDPC_VS value of 16.75, contrasting with the 24.13 of cv. Messire (ANOVA, $p<0.05$) (Fig. 1a). The ANOVA

Fig. 1 Frequency distributions for the five traits AUDPC_VS (**a**), AUDPC_RWCS (**b**), RWCS$_F$ (**c**) AUDPC_RWCL (**d**) and RWCL$_F$ (**e**) in P665 × cv. Messire RIL population. Values for both parents are indicated by *arrows*



revealed that the variation in AUDPC_VS among the RIL families was also significant ($p<0.001$), and a high value for broad sense heritability (h^2) was observed for this trait ($h^2= 0.702$) (Table 1). Furthermore, transgressive RIL lines with increased susceptibility were identified, as it is shown by the frequency distribution (Fig. 1a).

AUDPC_RWCS and AUDPC_RWCL represented the variation on RWC in soil and leaves along the time course whereas RWCS$_F$ and RWCL$_F$ represented the values for these traits on the last time point sampled, corresponding to terminal water stress.

Parental lines, showed an average AUDPC_RWCS value of 965.8 in the case of P665 and 830.8 of cv. Messire (Fig. 1b). The average RWCS$_F$ on the last time point observed for both parentals was 32.9 % for P665 and 20.0 % for cv. Messire (Fig. 1c). However, no significant differences were found between P665 and cv. Messire for AUDPC_RWCS ($p=0.519$) or RWCS$_F$ ($p=0.051$).

On the other side, the average AUDPC_RWCL for P665, characterised as drought tolerant on previous experiments (data not shown), was higher ($p<0.05$) than the one observed for cv. Messire (267.0 vs 276.2, Fig. 1d). At the end of the water stress period cv. Messire, which showed a moderate susceptibility to drought, displayed an average RWCL$_F$ value of 81.7 % whereas for P665 was of 90.0 % (Fig. 1e).

Variation in AUDPC_RWCS, AUDPC_RWCL, RWCS$_F$ and RWCL$_F$ among the RIL families was found to be highly significant according to ANOVA ($p<0.001$). Transgressive RIL lines with increased adaptation and susceptibility were identified for RWCS$_F$, RWCL$_F$ and AUDPC_RWCS. In addition, no transgressive lines with increased susceptibility were observed for the traits AUDPC_VS and AUDPC_RWCL (Fig. 1). Broad sense heritabilities (h^2) were high in all cases (Table 1).

Table 1 Heritability of the different traits scored in the RIL population

Trait	h^2
AUDPC_VS	0.702
AUDPC_RWCS	0.847
RWCS$_F$	0.856
AUDPC_RWCL	0.944
LRWC$_F$	0.697

h^2 broad sense heritability

The correlation observed between traits was found to be high for all traits except AUDPC_RWCL and AUDPC_VS ($r=0.391$) but significant for all of them ($p<0.001$) (Table 2).

QTL Analysis

Quantitative trait loci analysis revealed a total of ten QTLs associated with drought adaptation traits. Two QTLs were associated with the variable AUDPC_VS (*audpc_vs-1*, *audpc_vs-2*), four with RWCS$_F$ (*audpc_rwcs-1*, *audpc_rwcs-2*, *rwcs*$_F$*-1* and *rwcs*$_F$*-2*), and four with RWCL$_F$ (*audpc_rwcl*, *rwc*$_F$*l-1*, *rwcl*$_F$*-2* and *rwcl*$_F$*-3*) (Fig. 2). Genomic positions, LOD scores and additive effects of each QTL are shown in Table 3.

The QTLs explained individually from 9 to 33 % of the phenotypic variation depending on the variable and altogether from 20 to 57 % using MIM approach. Alleles conferring higher values of RWCS$_F$/L$_F$ and AUDPC_RWCS/L, or lower values of AUDPC_VS, and thus adaptation to water stress, were originated from P665 in case of all the QTLs except *audpc_rwcs-1*, *rwcs*$_F$*-1*, *audpc_rwcl* and *rwcl*$_F$*-1* for which the alleles promoting adaptation came from cv. Messire. Significant pairwise epistatic interactions among the QTLs for RWCS$_F$ was found in MIM and incorporated to the model.

Discussion

QTLs have been mapped for a wide range of agronomic traits in pea, including biotic and abiotic stresses. QTLs for incomplete and complete resistance have been detected for the most important diseases affecting pea (see Rubiales et al. 2009a as a review) and for adaptation to abiotic stress such as winter frost and frost damage (Lejeune-Hénaut et al. 2008; Dumont et al. 2009). However, to the best of our knowledge, no previous study has addressed the genetics of adaptation to drought in pea or reported QTLs for this trait. For the first time in this work, the genetics and heritability of drought adaptation in pea is analysed and the main zones of the genome associated with water stress adaptation have been identified. Differences between parental genotypes P665 and cv. Messire regarding water stress adaptation made it possible to assess visual symptoms along a water stress time course and analyse segregation of RWCS/RWCL and AUDPC_VS within the RILs.

Polygenic nature of drought adaptation has been pointed out in different studies in other crops based on a number of parameters associated with this trait (Bartels and Sunkar 2005; Cattivelli et al. 2008). The continuous distribution observed in our study within the RIL population for drought adaptation in pea as well as the identification of ten putative QTLs explaining from low to moderate percentage of the phenotypic variation also suggests a polygenic control of drought adaptation in pea.

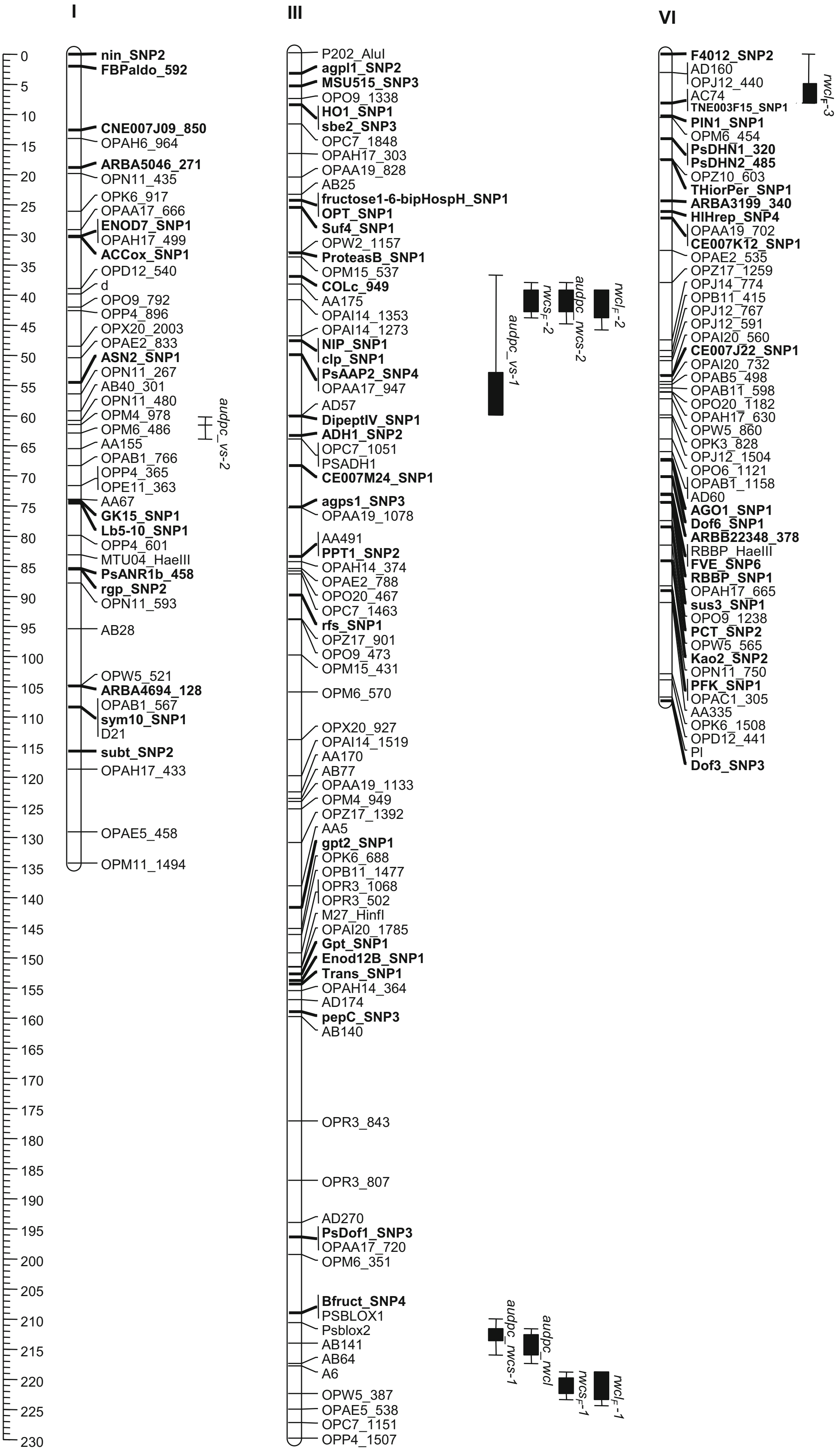
Once water was withheld, the levels of soil water content in the pots, decreased gradually along the time course, mimicking field situations (Quach et al. 2014). This method based in water withdrawal has been applied in several works (Gong et al. 2010; Quach et al. 2014). Furthermore, the reduction on the irrigation levels like in Turyagyenda et al. (2013) allows a gradual reduction of the soil water levels which mimics field conditions. The authors propose that this reduction is around 10 % every day. In our study, the reduction on soil water content was of 5 % daily, which would recreate mild water stress field conditions. RWCS has been reported to be useful for phenotyping under drought stress either by itself (Granier et al. 2006) or in combination with other measurements (Aguirreazabal et al. 2006). In wheat, RWCS has found to be related with water spectral indices in different genotypes (Gutierrez et al. 2010), although RWCS has never been used as a parameter to discriminate between tolerant or susceptible genotypes till now. Our study uses RWCS as a suitable trait for assessing quantitative drought adaptation in pea and reports the genomic regions associated with the character. Furthermore, monitoring RWCS allowed us to mimic water stress conditions of previous experiments as well as controlling water availability along the entire time course. RWCS$_F$ had a significant and strong negative correlation with AUDPC_VS which reflects the fact that plants showed more symptoms when water availability in soil was lower.

RWCL has been previously reported as a valuable and appropriate index to assess drought adaptation in a number of crops (Hunt et al. 1987; Tripathy et al. 2000; Siddique et al. 2000; Gutierrez et al. 2010). In pea subjected to drought stress, sensitive genotypes have been found to be more affected by the decline in RWCL than tolerant ones (Upreti et al. 2000). Despite other parameters such as leaf water potential and osmotic adjustment that have been traditionally used to assess plant water status as well as water and solutes transport in the soil–plant–atmosphere continuum (Kramer 1988), only

Table 2 Pearson's correlation coefficients among the traits assessed

Trait	AUDPC_VS	AUDPC_RWCS	RWCS$_F$	AUDPC_RWCL
AUDPC_RWCS	−0.620***			
RWCS$_F$	−0.598***	0.938***		
AUDPC_RWCL	−0.391***	0.614***	0.457***	
RWCL$_F$	−0.580***	0.780***	0.665***	0.803***

***$p<0.001$, significant differences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OPAH6_964
ARBA5046_271
OPN11_435
OPK6_917
OPAA17_666
ENOD7_SNP1
OPAH17_499
ACCox_SNP1
OPD12_540
d
OPO9_792
OPP4_896
OPX20_2003
OPAE2_833
ASN2_SNP1
OPN11_267
AB40_301
OPN11_480
OPM4_978
OPM6_486
AA155
OPAB1_766
OPP4_365
OPE11_363
AA67
GK15_SNP1
Lb5-10_SNP1
OPP4_601
MTU04_HaeIII
PsANR1b_458
rgp_SNP2
OPN11_593
AB28
OPW5_521
ARBA4694_128
OPAB1_567
sym10_SNP1
D21
subt_SNP2
OPAH17_433
OPAE5_458
OPM11_1494
audpc_vs-2
P202_AluI
agpl1_SNP2
MSU515_SNP3
OPO9_1338
HO1_SNP1
sbe2_SNP3
OPC7_1848
OPAH17_303
OPAA19_828
AB25
fructose1-6-bipHospH_SNP1
OPT_SNP1
Suf4_SNP1
OPW2_1157
ProteasB_SNP1
OPM15_537
COLc_949
AA175
OPAI14_1353
OPAI14_1273
NIP_SNP1
clp_SNP1
PsAAP2_SNP4
OPAA17_947
AD57
DipeptIV_SNP1
ADH1_SNP2
OPC7_1051
PSADH1
CE007M24_SNP1
agps1_SNP3
OPAA19_1078
AA491
PPT1_SNP2
OPAH14_374
OPAE2_788
OPO20_467
OPC7_1463
rfs_SNP1
OPZ17_901
OPO9_473
OPM15_431
OPM6_570
OPX20_927
OPAI14_1519
AA170
AB77
OPAA19_1133
OPM4_949
OPZ17_1392
AA5
gpt2_SNP1
OPK6_688
OPB11_1477
OPR3_1068
OPR3_502
M27_HinfI
OPAI20_1785
Gpt_SNP1
Enod12B_SNP1
Trans_SNP1
OPAH14_364
AD174
pepC_SNP3
AB140
OPR3_843
OPR3_807
AD270
PsDof1_SNP3
OPAA17_720
OPM6_351
Bfruct_SNP4
PSBLOX1
Psblox2
AB141
AB64
A6
OPW5_387
OPAE5_538
OPC7_1151
OPP4_1507
audpc_vs-1
rwcs$_F$-2
audpc_rwcs-2
rwcl$_F$-2
audpc_rwcs-1
audpc_rwcl
rwcs$_F$-1
rwcl$_F$-1
F4012_SNP2
AD160
OPJ12_440
AC74
TNE003F15_SNP1
PIN1_SNP1
OPM6_454
PsDHN1_320
PsDHN2_485
OPZ10_603
THiorPer_SNP1
ARBA3199_340
HlHrep_SNP4
OPAA19_702
CE007K12_SNP1
OPAE2_535
OPZ17_1259
OPJ14_774
OPB11_415
OPJ12_767
OPJ12_591
OPAI20_560
CE007J22_SNP1
OPAI20_732
OPAB5_498
OPAB11_598
OPO20_1182
OPAH17_630
OPW5_860
OPK3_828
OPJ12_1504
OPO6_1121
OPAB1_1158
AD60
AGO1_SNP1
Dof6_SNP1
ARBB22348_378
RBBP_HaeIII
FVE_SNP6
RBBP_SNP1
OPAH17_665
sus3_SNP1
OPO9_1238
PCT_SNP2
OPW5_565
Kao2_SNP2
OPN11_750
PFK_SNP1
OPAC1_305
AA335
OPK6_1508
OPD12_441
Pl
Dof3_SNP3
rwcl$_F$-3

◀ **Fig. 2** Pea genetic linkage map of the groups I, III and VII, where the QTLs related to drought were located, constructed from a population formed by 98 $F_{6:7}$ recombinant inbred lines (RILs) derived from the cross between the *P. sativum* subsp. *syriacum* accession P-665 and the *P. sativum* subsp. *sativum* cv. Messire. *Bar positions* indicate locations of quantitative trait loci: outer and inner interval corresponding to 1-LOD and 2-LOD support interval are indicated as a *full box* and a *single line*, respectively

RWCL takes into account the possible effect of both leaf water potential and osmotic adjustment. Thus, RWCL gives us an idea of how water and solutes move through the plant. A high negative correlation was also found for RWCL$_F$ and AUDPC_VS.

It has been reported that a decrease in RWCL can be a main factor resulting in reduced growth in response to osmotic stress in pea (Alexieva et al. 2001). Our results are in agreement with these, as RWCL was a suitable parameter to evaluate drought adaptation in our RIL population and parents and allowed the identification of the genomic regions associated with the trait. In our study RWCS$_F$ and RWCL$_F$ were highly correlated and two out of the three QTLs controlling RWCL$_F$ co-localised with QTLs for RWCS$_F$, suggesting a common genetic control which could be explained by their relation with the water transport along the plant.

Strong correlation between AUDPC_VS and RWC$_F$ in soil and leaves, respectively, points out the visual scale as a proper tool to assess different aspects and traits related to drought adaptation. Accordingly, correlation was higher when we were referring to the last point of the time course of both RWCS and RWCL (RWCS$_F$ and RWCL$_F$) instead of their respective AUDPC values, as the symptoms observed were more intense at the end of the drought period, when there is less water available in soil and plant tissues.

In the RIL population, the distribution was skewed towards lower values of RWCS$_F$/L$_F$ and AUDPC_RWCS/L, with many families showing lower values of these traits than cv. Messire. This fact suggested the existence of some QTLs that had alleles promoting high RWCL and RWCS under water stress also in the susceptible cv. Messire. These more susceptible lines in this RIL population would be those lacking favourable alleles at four of the QTLs associated with high relative water content (*audpc_rwcs-1*, *rwcs*$_F$*-1*, *rwcl*$_F$*-1* and

Table 3 QTLs in the RIL population derived from cross P665 by cv. Messire

Trait[a]	Linkage group	QTL	Flanking markers	Peak[b]	LOD[c]	Add[d]	R^2[e]
AUDPC_VS	III	*audpc_vs-1*	OPAA17_947/AD57	57.12	3.38	−0.69	10.94
	I	*audpc_vs-2*	OPM4_978/OPM6_486	61.56	3.32	−0.62	8.86
Total							19.80
AUDPC_RWCS	III	*audpc_rwcs-1*	Psblox2/AB141	212.80	11.88	−53.77	31.29
	III	*audpc_rwcs-2*	OPAI14_1353/OPAI14_1273	40.96	5.30	37.27	13.77
Total							45.06
RWCS$_F$	III	*rwcs*$_F$*-1*	A6/OPW5_387	221.04	12.24	−4.15	32.89
	III	*rwcs*$_F$*-2*	AA175/OPAI14_1353	40.42	6.94	3.07	19.64
		rwcs$_F$*-1*×*rwcs*$_F$*-2*				−1.84[f]	4.77
Total							57.30
AUDPC_RWCL	III	*audpc_rwcl*	AB141/AB64	215.21	7.36	−5.56	25.56
Total							25.56
RWCL$_F$	III	*rwcl*$_F$*-1*	OPW5_387/OPAE5_538	223.59	8.01	−6.96	24.02
	III	*rwcl*$_F$*-2*	OPAI14_1353/OPAI14_1273	40.96	4.85	4.78	11.37
	VI	*rwcl*$_F$*-3*	OPJ12_440/AC74	7.33	3.73	4.46	9.01
Total							44.40

[a] Traits assessed: AUDPC_VS, area under progress curve calculated for the mean values of the visual AUDPC_VS along the drought time course; AUDPC_RWCS, area under progress curve calculated for the mean values of RWCS along the 15-day time course; AUDPC_RWCL, area under progress curve calculated for the mean values of RWCL along the water stress time course; RWCS$_F$, soil relative water content 15 days after watering withdrawal; RWCL$_F$, mean value of the RWCL for a 22 % of average RWCS

[b] Peak: QTL position (cM)

[c] LOD: peak LOD score from CIM

[d] Add: the additive effect from CIM (AUDPC_RWCL) or MIM (AUDPC_RWCS, RWCS$_F$, AUDPC_VS, LRWC$_F$)

[e] R^2 (%): proportion of phenotypic variance explained by the respective QTL (for AUDPC_RWCS, RWCS$_F$, AUDPC_VS and LRWC$_F$, the partial R^2 of each QTL was estimated from MIM)

[f] The value refers to the epistatic effects. Epistatic interaction between QTLs for RWCS$_F$ was found significant in multiple interval mapping (MIM) and included in the model

audpc_rwcl) derived from cv. Messire. In fact, we had observed in previous studies (data not shown) that cv. Messire preserved a moderately high relative water content under water stress when compared with genotypes highly affected by drought, indicating that cv. Messire was moderately susceptible. Our present results support the hypothesis that genes conferring high RWCS/L in cv. Messire and in P665 were different. Some transgressive RIL families with higher RWCS$_F$/L$_F$ and AUDPC_RWCS values than both parents were identified. These RIL families which showed a higher RWC than P665 might possess favourable alleles for all adaptation QTLs. This would be in agreement with the negative epistatic effect observed in the case of QTLs *rwcs*$_F$*-1* and *rwcl*$_F$*-2* given the fact that the complementary favourable alleles were in repulsion phase.

Through our study, we were able to clearly distinguish the genomic regions related to drought adaptation in each parental line. Thus, the QTLs promoting adaptation from cv. Messire were all located in the distal part of LGIII. By contrast, QTLs for drought adaptation from P665 (*audpc_rwcs-2*, *rwcs*$_F$*-2*, *rwcl*$_F$*-2* and *rwcl*$_F$*-3*) were located in three different regions, one at the beginning of LGIII and two in LGI and LGVI.

Although the QTLs identified explained a high proportion of phenotypic variation for most of the traits, they could not explain all the observed variation. Nevertheless, it is possible that the parents possess some common adaptation genes and that a QTL analysis in RILs from cross with an accession more susceptible than cv. Messire may allow detection of additional genes which might be governing high RWCS/L within the segregating population. In addition, the measurement of additional parameters and the use of other assessment tools for traits related with drought adaptation may allow us to better unravel the complexity of the genetic networks implied on the physiological responses in the plant.

The population used in our study had been previously used to identify QTLs for other traits that could be related to drought resistance such as root lenght, blooming date or aerial plant biomass (Fondevilla et al. 2011). Three QTLs associated with RWCS/L (*rwcl*$_F$-1, *rwcl*$_F$*-3* and *rwcs*$_F$*-1*) were in the same genomic region as two QTLs controlling resistance to *Orobanche crenata* in this same RIL population (Fondevilla et al. 2011). Thus, *rwcl*$_F$*-1* and *rwcs*$_F$*-1* were mapped exactly in the same region of LGIII as the QTLs *n°br03_2*, and *rwcl*$_F$*-3* was in the same region of LGVI as the QTLs *n°br04_1*. Both *n°br04_1* and *n°br03_2* are QTLs for *O. crenata* incomplete resistance under field conditions. Interestingly, the parasitic plant *O. crenata* obtains nutrients, but also water from its pea host. Therefore, water and solutes loss produced by drought and by *O. crenata* attack could be controlled by similar genomic regions or maybe these genomic regions also control phenology, root morphology and other traits that contribute to reduction of both drought and broomrape damages. Supporting these hypotheses, the alleles promoting higher RWC and resistance to *O. crenata* would come from cv. Messire in the QTLs *rwcl*$_F$*-1*, *rwcs-1* and *n°br03_2*, whereas the adaptation to drought and the resistance to *O. crenata* associated to the QTL *rwcl*$_F$*-3* and *n°br03_2*, respectively, would be conferred by P665.

Furthermore, the QTLs *rwcl*$_F$*-1* and *rwcs*$_F$*-1* were located in the confidence interval of the QTL *dfIII.1*, associated to earliness (Fondevilla et al. 2011), which would also allow avoiding seasonal drought stress (Forrest and Miller-Russhing 2010). Although blooming date was obtained in field conditions, earliness could be meaningful as differences in growing patterns between lines could be due to a different drought adaptation/sensibility level. Also, knowing this could be helpful in the process of selecting material both for adaptation and escape to drought. Other QTLs such as *MpIII.1_DRseedl*, both associated with incomplete resistance to *Didymella pinodes* and *Psy1*, associated with *Pseudomonas syringae* pv. *syringae* resistance, are located in this region (Fondevilla et al. 2011, 2012), which could mean that these genes are involved both in biotic and abiotic stress. In this case, the alleles promoting drought adaptation came from P665, whereas cv. Messire conferred the resistance to *D. pinodes* and the earliness.

Among the QTLs associated with RWC derived from P665, *rwcl*$_F$*-2* (LGIII), *rwcs*$_F$*-2* (LGIII) and *audpc_rwcs-2* (LGII) explained the 11.37, 19.64 and 13.77 % of the phenotipic variability for this trait, respectively. In addition, these QTLs were located in the same genomic region as a QTL (*rl3*) associated with higher root length in cv. Messire, but also with another QTL, *dfIII.2*, related to earliness in flowering tested under field conditions in a previous study (Fondevilla et al. 2011). Furthermore, this genomic region was found to be associated with the QTLs *MpIII.3_DRl_05*, *MpIII.3_DRst_05*, *MpIII.3_DRl_06* and *MpIII.3_DS_06* all of them related to *D. pinodes* resistance (Fondevilla et al. 2011). It seems that precocity and *D. pinodes* resistance are somehow related in field conditions, and it is possible that these QTLs are more related to flowering than associated to genetic resistance to *D. pinodes* per se. Both QTLs for earliness and *D. pinodes* resistance came from P665.

The relationship between RWCS/RWCL and roots has been previously observed in rice, where the genes controlling resistance to drought and root morphology traits were detected in the same region (Yue et al. 2005). In fact, root morphology is widely known as a trait related with drought avoidance (Grzesiak et al. 1997; O'Toole and Moya 1978; Ludlow 1989). Selection for deep and extensive root system has been advocated to increase productivity of food legumes under moisture deficit conditions as it can optimise the capacity to acquire water. Turner et al. (2001) identified rooting depth and density as a main drought avoidance trait in grain legumes for use in terminal-drought environments. Therefore, it was highly significant that SRWC and root length QTLs were located in the same region in our study.

SSRs and SNPs are robust tools for marker-assisted selection (MAS). In our study, *rwcs*$_F$*-1* was associated to the SSR marker A6 whereas *rwcl*$_F$*-2*, *audpc_rwcs-2* and *rwcs*$_F$*-2* were in the vicinity of the SSR marker AA175. In addition, *rwcl*$_F$*-3* and *audpc_vs-1* were flanked on one side by AC74 and AD57, respectively, and *audpc_rwcl* was flanked by AB141/AB64, all of them SSR markers which can be used to detect these QTLs. SSR markers are locus-specific, easy to score due to the absence of similar sized interfering fragment, low sensitive to reaction conditions and reproducible. Therefore, the SSR markers A6, AA175, AC74, AD57, AB141 and AB64 associated with the drought adaptation QTLs could be useful for MAS in drought adaptation breeding programs.

Gene-based markers *audpc_vs-1*, *audpc_rwcs-1* and *rwcs*$_F$*-1* were also identified in our study and can be also used as robust markers to select these QTLs in MAS. In the case of *audpc_vs-1*, it was flanked by the SNPs PsAAP2_SNP4 and DipeptIV_SNP1 which correspond to the proteins *Aminoacid permease II* and *Dipeptidyl peptidase IV like-protein*, related with general metabolic process such as amino acid transport and proteolysis. On the other hand, *audpc_rwcs-1* was flanked by *Psblox2*, which is an SSR corresponding to a lipoxygenase, enzyme whose genetic expression is related to wounds, defence or water deficit response (Bell and Mullet 1991; Aubert et al. 2006) and has interestingly found to be associated to drought stress tolerant peanut genotypes (Kottapalli et al. 2009). We suggest *Psblox2* as a candidate gene for drought adaptation in pea, although additional studies are needed to validate or reject this hypothesis.

Finally, both *audpc_rwcl* and *rwcs*$_F$*-1* would be also in the LOD2 region of the "*Le*" gene (Lester et al. 1997; Bordat et al. 2011), which presence in this zone would be quite significant regarding drought adaptation. This gene encodes a Gibberelin 3P-hydroxilase, an enzyme related with activation of the plant hormones gibberellins (GAs) and traditionally associated with growth regulation (Lange et al. 1999), but also found to be implied in stress protection (Vettakkorumankankav et al. 1999) and modulation (Alonso-Ramírez et al. 2009). Hormones such as ethylene, salicylate, jasmonate and abscisic acid (ABA) act synergistically or antagonistically to regulate plant responses to pathogens and abiotic stress factors (Rao et al. 2000, 2002; Borsani et al. 2001; Turner et al. 2002; Xiong et al. 2002). Thus, the presence of QTLs related to drought stress in this region would be in agreement with the QTLs described in previous studies and also support the biotic and abiotic stress connections on the gene level. Furthermore, *Le* could be a good candidate gene for drought adaptation. However, further studies are needed to confirm the role of *Le* gene in drought adaptation.

The present study identified ten QTLs from pea which are related to different aspects of drought stress in the cross P665 × Messire. Despite further validation of these results should be considered it provides a set of robust markers A6, AA175, AC74, AD57, AB141, AB64, *Psblox2*, PsAAP2-SNP4 and DipeptIV_SP1 linked to these QTLs that could possibly facilitate drought adaptation gene transfer into elite pea cultivars in MAS schemes. The amplification profiles of the alleles of these loci which are associated with increased drought adaptation are shown in Online resource 4.

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Conflict of Interest The authors declare that they have no conflict of interest.

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