Development of molecular markers linked to QTL/genes controlling Zn efficiency

Hasan Pinar1 · Cansu Bulbul2 · Duran Simsek3 · Mostafakamal Shams4 · Nedim Mutlu2 · Sezai Ercisli4

Received: 25 July 2021 / Accepted: 30 September 2021 / Published online: 23 October 2021
© The Author(s), under exclusive licence to Springer Nature B.V. 2021

Abstract
Background Zinc (Zn) deficiency is a widespread problem in reducing the yield and quality of crop plants worldwide. It is important to utilize molecular markers linked to Zn efficiency to develop high Zn efficient cultivars in pepper (Capsicum annuum L.).

Methods and results In present study, genetic map was constructed using a F2 populations derived from C. annuum L. (Alata 21A) × C. frutescens L. (PI 281420) cross. The quantitative trait locus (QTLs) for Zn efficiency were mapped using F2:3 population. A genetic map with 929.6 cM in length and 12 linkage groups were obtained using 62 markers (31 sequence-related amplified polymorphism (SRAP), 19 simple sequence repeat (SSR) and 11 random amplified polymorphic DNA (RAPD) markers). The 41 linked QTLs related with nine (9) Zn efficiency characters were mapped on linkage groups. Results suggest that selecting plants for tolerance to Zn deficiency are expected to be rather responsive among segregating populations for breeding and developing Zn efficient genotypes in pepper.

Conclusions The molecular markers are expected to aid selection and expedite breeding peppers resistant to Zn deficiency in soils low for available Zn contents.

Keywords Pepper · Zn efficiency · Genetic mapping · SSR · SRAP · RAPD

Introduction
Zinc (Zn) deficiency causes important disturbances in the growth and development of plants due to the large diversity of essential cellular functions and metabolic pathways [1]. Zn plays an important role in the control of phosphor (P) absorption in higher plants, it prevents excessive P uptake by roots and the transport of P from roots to leaves and imbalance of ions induced by reduction in K (potassium) concentration [2, 3]. Zn deficiency leads to severe symptoms such as interveinal chlorosis in leaves, reddish-brown or bronze tints, epinasty, internode shortening, inward curling of leaf lamina, and decrease in leaf size [3]. However, Zn-efficient genotypes can be able to utilize Zn more effectively than other (less efficient) genotypes and they have normal growth and production capacity, even in soils with Zn deficiency but do not necessarily have the highest Zn content in tissue or grain [4].

The concentration of Zn in plants is influenced by complex genetics and is also affected by environmental factors. Quantitative trait locus (QTL) analysis of complex traits can find a relation between markers and traits that can explain
the genetic mechanism of complex traits. The use of markers linked to QTL for tolerance to Zn deficiency may aid pepper breeders via marker-assisted selection (MAS) impose positive selection on tolerant cultivars and negative selection on sensitive cultivars with using.

Zn uptake has a complex inheritance. The 9 QTLs that govern Zn uptake in Barley [5] where one QTL control the Zn uptake by roots and its transport from the root to the shoots, and two QTLs control Zn translocation in plants and one QTL also controls the Zn content in the stem at flowering stage of the barley. There are four QTLs controlling Zn and P content in seeds of wheat [6].

Pepper is one of the main vegetable crops grown worldwide and is categorized into 25 species and displays a wide range of genetic diversity. It is sensitive to Zn deficiency [2, 7, 8], but there was a significant variations among pepper genotypes to Zn deficiency [9]. The presence of wide genetic variation indicates that Zn-efficient genotypes can be developed. There is no report for development of Zn efficient pepper due probably to existence of different mechanism of tolerance to Zn deficiency, and complex inheritance with low narrow sense heritability make it harder to make genetic gains per selection cycle. The objective of this study was to identify and map genes/QTLs governing Zn efficiency.

Materials and methods
Plant materials

The mapping population was derived from a cross between Capsicum annuum L. (Alata 21A) and Capsicum frutescens L. (PI 281420) as parents. The pure pepper line Alata 21A was obtained from pepper breeding programs conducted by Alata Horticultural Research Station (Mersin/Turkey) and whereas the genotype PI 281420 was supplied from AVRDC (World Vegetable Center). The F1 plant was selfed to produce F2 population (138 plants), and F2 families were created by selfing F2 plants. Initially, 455 F2 plants were planted in pods filled with extreme Zn deficient soil and tested against Zn deficiency. At the beginning of flowering, symptoms of Zn deficiency were scored according to the 1–5 scale in order to identify the most sensitive and the most resistant individuals. The plants were not harvested at this stage but switched to normal fertilization regime and F3 seeds were harvested from these plants. The leaf samples were collected for DNA extraction from the F2 plants during the period when they showed healthy growth under the normal fertilization regime. The 138 genotypes of the 455 F2 plants were selected for use in mapping. For this purpose, among 455 F2 plants 80 plants with the highest Zn deficiency symptoms (most sensitive) that was rated between 4.5 to 5.0 and 100 plants with the lowest Zn deficiency symptoms that were rated between 1.0 to 1.5 (showing the highest Zn efficiency) were selected for further evaluation.

Trait evaluation

Two parallel experiments were established, with and without Zn fertilization to be able to compare effect of Zn on parents, F1, and F2, family means. The experiment was set up with three replications for the parents and F1 plants according to the randomized blocks trial design and one seedling was planted in each pot. Each replication consisted of four plants of the parents and F1, and each F2 family (12 plants/family). The experiment was repeated twice, in 2013 (fall), and 2014 (spring). The seeds were sown in peat and then the seedlings planted in 2 L pots filled with a soil type, which is formed on the alluvial main material in Eskişehir-Sultanönü region known to have extremely low Zn content. The soil texture was clay loam with 0.14 mg kg−1 Zn (extractable Zn with DTPA), pH 7.6, 20% lime, and 0.96% organic matter contents. The soil was fertilized only at the beginning of the experiment before seedling planting with 200 mg kg−1 of Ca(NO3)2·4H2O, 100 mg kg−1 of KH2PO4 (125 mg kg−1 K) and 2.5 mg kg−1 of FeEDTA. Treatments were conducted at two levels of Zn [control (0) and 7.5 mg kg−1 of ZnSO4·7H2O] which was thoroughly mixed with the soil. After planting, the pepper plants were directly kept under natural light conditions according to a completely randomized design. During the experiment, relative humidity was 65% and the temperature was regulated at 25/20 °C (day/night). Also, irrigation was performed when the field capacity of the pots reached 70% and the plants were irrigated with tap water to maintain the moisture level at field capacity.

Determination of Zn content

The plant samples were collected from the plants grown with and without Zn supply. The samples of roots and leaves from parents, F1, F2, and F3 plants were oven-dried for 48 h at 70 °C. Then they were ground and passed through a mesh 1 mm in size. The Zn content was determined by inductively coupled plasma-atomic emission spectroscopy (ICP) after wet digestion with nitric acid and hydrogen peroxide [10].

DNA extraction, marker analysis and map construction

Pepper genomic DNA was isolated from young leaves collected from 138 F2 plants, and the parents, using the modified cetyltrimethylammonium bromide (CTAB) method as reported by Shams [11].
Simple sequence repeat (SSR) analysis

The SSR markers that have the highest polymorphism were first screened from the co-dominant marker list that formed the C. frutescens × C. annum integrated map. The 138 F₂ individuals were genotyped by markers (corresponding to 12 chromosomes) and 24 markers that had the highest polymorphism were selected for mapping by sequence-related amplified polymorphism (SRAP) and random amplified polymorphic DNA (RAPD) techniques.

According to sequences and map information was provided on Solgenomics.net [12], 60 primer pairs were tested with parents and among them, 24 primers (Online Resource 1) with the highest polymorphism were selected, and 138 plants of F₂ populations were evaluated.

Sequence-related amplified polymorphism (SRAP) analysis

In the SRAP analysis studies, 208 SRAP primer combinations were used to screen for parental polymorphism and 31 primer combinations (Online Resource 1) were selected. The parents and the F₂ population were genotyped with 31 SRAP primer combinations. They were scored as the dominant marker. PCR amplification was conducted as reported by Li and Quiros [13].

Random amplified polymorphic DNA (RAPD) analysis

For RAPD analysis, 12 different 10-mer RAPD primers (Online Resource 1) were used and PCR amplification conditions were performed as reported by Williams et al. [14]. Polymorphisms were first identified between the parents with 150 RAPD primers and 12 primers which had the highest polymorphic results were selected. The parents and F₂ population were genotyped using these polymorphic primers and scored as the dominant marker.

Electrophoresis and gel imaging

The 3 µL loading buffer [20 mL glycerol (40%), 30 mL sterile water, 0.05 g bromophenol blue] was added into PCR products that was loaded on 2% agarose gel for SRAP and RAPD, and run under 115 V for 3.5 h. For SSR markers, PCR products were run on 3% high-resolution agarose gel.

Map construction and QTL detection

The SRAP and RAPD markers were scored as dominant and the SSRs as codominant markers. The linkage groups were created by JOINMAP 4.1 [15] software and the MapQTL 4.0 package [16] was used to perform QTL mapping analysis of “Kruskal Wallis (KW)”, “interval mapping (IM)”, MQM and “composite interval mapping (rMQM)”.

Results

Twelve linkage groups (LGs) were created with 163 polymorphic markers using the JoinMap 4.1 software. The LGs (Table 1) were created using 31 SRAP, 19 SSR, and 11 RAPD markers. Based on the results, a map of 929.6 cM length was obtained, with a total of 62 polymorphic bands and 12 LGs. The length of each LGs is given in Table 1. The average distance between the markers was 14.99 cM. The SSR markers were used to assign the LGs similar to Wu et al. [12] in Solgenomics.net for the population of C. annum × C. frutescens.

QTL mapping

QTLs were mapped in the MapQTL.6 software after the LGs was created by JoinMap 4.1 software. The quantiative data obtained from replicated F₂:3 families under Zn deficiency were mapped on LGs using “Kruskal Wallis” and “Internal Mapping” analyzes. The QTLs for nine traits and their mapped chromosome region are presented in Table 2 and Fig. 1.

Table 1 Distribution of markers on linkage groups developed from C. annum × C. frutescens F₂ population

| Chromosome (Chr) | Chr1 | Chr2 | Chr3 | Chr5 | Chr6 | Chr8 | Chr9 | Chr10 | Chr11 | ChrX | ChrXX | ChrXXX | Total |
|------------------|------|------|------|------|------|------|------|-------|-------|------|-------|--------|-------|
| Total            | 5    | 3    | 3    | 7    | 5    | 8    | 7    | 7     | 5     | 4    | 5     | 3      | 31     |
| SSR              | 2    | 2    | 1    | 1    | 1    | 5    | 3    | 2     | 2     | –    | –     | –      | 7      |
| SRAP             | 3    | –    | 2    | 5    | 3    | 3    | 3    | 4     | 1     | 3    | 4     | 1      | 15     |
| RAPD             | –    | 1    | –    | 1    | 1    | –    | 1    | 1     | 2     | 1    | 1     | 2      | 8      |
| Length (cM)      | 95.4 | 2.1  | 83.7 | 113.5| 59.3 | 95.5 | 114.4| 104.9 | 94.3  | 88.5 | 39.3  | 19.7   | 461.1  |
| Average interval (cM) | 19.1 | 7.03 | 27.6 | 16.2 | 11.9 | 11.9 | 16.3 | 14.9  | 18.9  | 22.1 | 7.86  | 6.6    | 14.99  |
Scores of Zn deficiency symptoms in the F3 population

Zn deficiency symptom scores were recorded once a week throughout a six week period, starting at the beginning of the flowering stage. The average scores of 12 plants of each family derived from 126 F2:3 populations was analyzed by the MapQTL software. The four QTLs were mapped on LGs throughout the six week period, starting at the beginning of the flowering stage. The average scores of 12 plants of each family derived from 126 F2:3 populations was analyzed by the MapQTL software. The four QTLs were mapped on LGs.

1. **Scores of Zn deficiency symptoms in the F3 population**
   - **Trait Number**: 1
   - **Trait**: Scores of Zn deficiency symptoms in the F3 population
   - **QTL Symbol**: f3scorX.1
   - **Chr**: EM8ME7.270
   - **Position (cM)**: 0
   - **R^2 Direction**: PI 281420
   - **Marker**: PI 281420
   - **QTL Position**: 2.3

2. **Zn effectiveness in terms of total dry matter weight**
   - **Trait Number**: 2
   - **Trait**: Zn effectiveness in terms of total dry matter weight
   - **QTL Symbol**: tdmznefX.1
   - **Chr**: EM14ME1.480
   - **Position (cM)**: 73.02
   - **R^2 Direction**: PI 281420
   - **Marker**: PI 281420
   - **QTL Position**: 3.2

3. **Zn effectiveness in terms of plant length**
   - **Trait Number**: 3
   - **Trait**: Zn effectiveness in terms of plant length
   - **QTL Symbol**: plhtznefX.1
   - **Chr**: C2At1g44760
   - **Position (cM)**: 83.68
   - **R^2 Direction**: PI 281420
   - **Marker**: PI 281420
   - **QTL Position**: 6.2

4. **Zn effectiveness in terms of Zn concentration in total dry matter**
   - **Trait Number**: 4
   - **Trait**: Zn effectiveness in terms of Zn concentration in total dry matter
   - **QTL Symbol**: znconefX.1
   - **Chr**: GP20095
   - **Position (cM)**: 47.79
   - **R^2 Direction**: PI 281420
   - **Marker**: PI 281420
   - **QTL Position**: 5.1

5. **Zn effectiveness in terms of leaf dry matter weight**
   - **Trait Number**: 5
   - **Trait**: Zn effectiveness in terms of leaf dry matter weight
   - **QTL Symbol**: ldmwefX.1
   - **Chr**: EM14ME1.480
   - **Position (cM)**: 73.02
   - **R^2 Direction**: PI 281420
   - **Marker**: PI 281420
   - **QTL Position**: 5.2

6. **Scores of Zn deficiency symptoms in the F2 population**
   - **Trait Number**: 6
   - **Trait**: Scores of Zn deficiency symptoms in the F2 population
   - **QTL Symbol**: f2scorX.1
   - **Chr**: GP20095
   - **Position (cM)**: 47.79
   - **R^2 Direction**: PI 281420
   - **Marker**: PI 281420
   - **QTL Position**: 6.8

7. **Zn effectiveness in terms of Zn content in leaves**
   - **Trait Number**: 7
   - **Trait**: Zn effectiveness in terms of Zn content in leaves
   - **QTL Symbol**: lznctefX.1
   - **Chr**: OPAC10.330
   - **Position (cM)**: 33.78
   - **R^2 Direction**: PI 281420
   - **Marker**: PI 281420
   - **QTL Position**: 5.6

8. **Zn effectiveness in terms of Zn content in dry matter**
   - **Trait Number**: 8
   - **Trait**: Zn effectiveness in terms of Zn content in dry matter
   - **QTL Symbol**: tdnzncntX.1
   - **Chr**: EM14ME1.480
   - **Position (cM)**: 73.02
   - **R^2 Direction**: PI 281420
   - **Marker**: PI 281420
   - **QTL Position**: 9.9

9. **Zn effectiveness in terms of Root/Shoot Ratio**
   - **Trait Number**: 9
   - **Trait**: Zn effectiveness in terms of Root/Shoot Ratio
   - **QTL Symbol**: rsrefX.1
   - **Chr**: CAEMS060
   - **Position (cM)**: 40.66
   - **R^2 Direction**: PI 281420
   - **Marker**: PI 281420
   - **QTL Position**: 3.4

---

**Table 2** The list of QTLs and associated markers identified for Zn deficiency symptoms in pepper

| Trait Number | Trait name                                      | QTL Symbol | Chr     | Marker | QTL Position (cM) | R^2 Direction |
|--------------|-------------------------------------------------|------------|---------|--------|-------------------|---------------|
| 1            | Scores of Zn deficiency symptoms in the F3 population | f3scor1.1 | EM8ME7.270 | 0      | 2.3               | PI 281420     |
| 2            | Zn effectiveness in terms of total dry matter weight | tdmznef3.1 | EM6ME6.260 | 83.67  | 4.7               | PI 281420     |
| 3            | Zn effectiveness in terms of plant length        | plhtznef3.1 | EM6ME6.260 | 83.68  | 6.2               | PI 281420     |
| 4            | Zn effectiveness in terms of Zn concentration in total dry matter | znconef3.1 | BM59622 | 0      | 5.8               | –             |
| 5            | Zn effectiveness in terms of leaf dry matter weight | ldmwef3.1 | EM14ME1.480 | 73.02  | 4.5               | PI 281420     |
| 6            | Scores of Zn deficiency symptoms in the F2 population | f2scor1.1 | GP20095 | 47.79  | 6.8               | –             |
| 7            | Zn effectiveness in terms of Zn content in leaves | lznctef3.1 | OPAC10.330 | 33.78  | 3                 | PI 281420     |
| 8            | Zn effectiveness in terms of Zn content in dry matter | tdnzncnt3.1 | EM7ME6.200 | 88.54  | 3.8               | PI 281420     |
| 9            | Zn effectiveness in terms of Root/Shoot Ratio    | rsref1.1 | CAEMS060 | 40.66  | 3.4               | –             |

---

aThe parent which contributes to the increase in the numeric value of the trait

---

 Springer
Fig. 1 Distribution of SSR, SRAP and RAPD markers and Zn efficiency related QTLs on 12 linkage groups of pepper
Zn efficiency for total dry matter weight

Zn efficiency in terms of total dry matter (Leaf+stem+root) was calculated from 24 plants of each F₃ family (12 plants under Zn supplied and 12 plants under the Zn deficiency).

As shown in Online Resource 2, Alata 21A had 59% higher Zn efficiency when compared with PI 281420. The F₁ plants exhibited 81% Zn efficiency, exceeding parental averages (71.85%). This indicates that the tolerance to Zn deficiency is partially dominant. Even when Zn deficiency symptoms are mild, a significant decrease can occur in the dry matter of the plant. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to dry matter weight was decreased under Zn deficiency. However, total dry matter weight of the F₁ plant was higher than the parents. A wide variation was obtained from the parent, F₁ and F₃ plants regarding to their response to 

Zn efficiency for leaf dry matter weight

The leaves, stem, and root samples of the plants were harvested separately and analyzed. The distribution of Zn based on leaf dry matter weight is presented in Online Resource 2. The lowest Zn dry matter was observed in the PI 281420 and the highest in Alata 21A while F1 had a value close to the tolerant parent, indicating an incomplete dominance of the trait. The three QTLs were mapped on LGs 3, 8, and 11 for the trait and explained 15.3% of variance (Table 2 and Fig. 1). The three QTLs associated with total dry matter weight were also co-localized with the QTLs for the plant height.

Scores of Zn deficiency symptoms in the F₂ population

The six times scoring of Zn deficiency symptom of 455 F₂ plants was made according to the 1–5 scale, and the distribution of data from the final scoring was presented in Online Resource 2. Based on the results, some F₂ plants had more severe symptoms than the sensitive parent (PI 281420) and also some seedlings were more tolerant than the tolerant parent (Alata 21A) under Zn deficiency, indicating a transgressive segregation for both ends. As presented in Online Resource 2, it was demonstrated that Zn deficiency is partially dominant. Three QTLs were found for the symptoms in F₂, two on the LG8, and one on LG10. However, an additional QTL was determined for the Zn deficiency symptom scores of the F₃ population (Online Resource 2). Therefore, the QTLs on LGs8 and 10 confirmed that this trait is multigenic. The QTL on LG8 co-localized with QTLs for Zn concentration in total dry matter, Zn contents in leaves and dry matter. The tree QTLs explained 17.9% variance for the Zn deficiency symptom in the F₂ population (Table 2 and Fig. 1).
Zn efficiency for Zn content in leaves

The segregating populations showed variation for leaf Zn content. The three QTLs were mapped on LGs 6, 8, and 11, and the QTLs on LGs 8 and 11 were co-localized with QTLs for total dry matter and leaf dry matter weights, and Zn deficiency symptoms in F2. The three QTLs explained 14.8% variance of leaf dry matter (Online Resource 2 and Table 2 and Fig. 1).

Zn efficiency for Zn content in dry matter

A very wide variation (14.0–89.7%) was found in terms of Zn content in total dry matter (Online Resource 2). The F1 plants showed a higher value than both parents, indicative of a heterosis or epistatic effect. Furthermore, as for Zn content in stem and root, F1 plants showed the higher value than both parents, while in leaves they responded similar to sensitive parent.

The six QTL were mapped for this trait; two QTLs on LGX, two on LG8, one on LG9, and one on LG11. Although there were 3 QTL effective on Zn content of leaf dry matter, 6 QTL was determined in total content. The position of the QTLs located on the LGs 8 and 11 are close to each other. The six QTLs (Table 2 and Fig. 1) explain 33.2% of variation for Zn content in total dry matter.

Zn effectiveness in terms of root/shoot ratio

Root/shoot ratio is one of the most important parameters in plant growth. The water and nutrient uptake may be increased by enhanced root/shoot ratio. In the Zn deficiency of the soils, the Zn uptake will be increased by an increase in the amount of root and expansion of the root surface area. There was a large variation in the F3 population. The F1 plants has similar root/shoot ratio, similar to sensitive parent. The five QTLs were mapped on LGs 1, 5, 6, 10, and 11 (Table 2 and Fig. 1). The 5 QTLs explained 20.6% variance for the trait (Online Resource 2).

Discussion

The studies were carried out to elucidate QTL that control Zn content in seeds of Arabidopsis and beans [19–21]. The QTL effective on increasing the Zn content in seed of barely was identified in the short arm of the 2H chromosome [22]. On the other hand, 2 QTLs related to Zn concentration and content in Barley seeds were mapped on the short and long arm of the 2H chromosome [23]. In our pepper study, 5, 6, and 3 QTLs for Zn concentration in total dry matter, Zn content in total dry matter and leaf Zn content were determined, respectively (Table 2 and Fig. 1). However, in barley, a few QTLs for Zn concentration and content were found [5], demonstrated that 9 QTLs control Zn absorption in barley; one of them controls Zn absorption by roots or its transport from root to shoots, two QTLs control Zn translocation in shoot and one QTL controls Zn content in the stem at maturity stage. Furthermore, Peleg et al. [24] reported a significant positive correlation between wheat grain protein concentration and grain Zn content, and it was demonstrated that 3 out of 10 QTLs identified for grain protein concentration control Zn content. Genc et al. [25] demonstrated a significant negative correlation between shoot Zn concentration and shoot biomass in wheat, and two QTLs promoted healthy growth (provides Zn activity) of plants under Zn deficiency. Furthermore, it was reported that the effect of genotype × environment interaction on the Zn content in wheat grain was less than 16%, and 72% was influenced by heredity. In this study, QTL 3, 4, and 7 were determined leaves dry matter weight, total dry matter weight and plant height, respectively. And these QTLs are located in the same region on the LGs 3, 8, and 11. The co-localized QTLs can be transferred together. Similarly, in wheat, it was determined that 4 QTL controls Zn concentration in grain, and they were located on the same chromosomal region [6]. To determine the Zn deficiency tolerance in rice, the QTL mapping was conducted with the population derived from a cross between IR74 (sensitive) and Jalmagna (tolerant); four QTLs associated with plant death were identified, and one of them was also associated with leaf browning [26]. In this study, according to the scoring of Zn deficiency symptom, 3 QTL in the F2 population and 4 QTLs in the F3 populations were identified. The QTLs were located in the same regions on LGs 8 and 10. Therefore, differences in number of QTLs to control Zn concentration among plant species may have resulted from the differences in plant species and population types.

Based on the results, it was detected that 3 QTLs control Zn content of leaves and 6 QTLs in total control Zn content of total dry matter. These results are similar to those of El-Bendary et al. [27] who reported that 4 genes control Zn content of leaves in maize. Furthermore, Hartwig et al. [28] demonstrated that 3 genes control Zn content in leaf of F3 population from sensitive and tolerant parents in soybean. On the other hand, Genc et al. [29] found that only a non-dominant gene controls Zn activity in F2 and F3 populations in barley. The inconsistencies may be related to the plant genotype and mapping population.

In summary, in pepper Zn efficiency traits are polygenic and difficult to breed for. The QTLs for total dry matter weight, leaf dry matter weight, Zn content and concentration of leaf and total dry matter, plant height, Zn deficiency symptom in F2, and F3 population were mapped on pepper LGs. Therefore, the QTLs for Zn efficiency may aid molecular marker aided breeding of peppers for regions with low available soil Zn.
**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11033-021-06736-9.

**Acknowledgements** This work “Development of Molecular Markers Linked to Qtl/Genes Controlling Zn Efficiency” contains data presented to Çukurova University for Ph.D. dissertation thesis by Hasan PINAR.

**Author contributions** Conceptualization: Hasan PINAR, Nedim Mutlu; Methodology: Hasan PINAR, Nedim Mutlu; Formal analysis and investigation: Hasan PINAR, Nedim Mutlu; Writing—original draft preparation: Hasan PINAR, Nedim Mutlu; Writing—review and editing: Hasan PINAR, Nedim Mutlu, Cansu Bulbul; Funding acquisition: Duran Simsek; Resources: Hasan PINAR; Supervision: Hasan PINAR, Nedim Mutlu.

**Funding** This research was funded by TUBITAK, grant number TUBITAK TOVAG 1100107.

**Declarations**

**Conflict of interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Consent to participate** All the authors mentioned in the manuscript have agreed for authorship, read and approved the manuscript.

**Consent to Publish** All the authors give the consent for the publication of identifiable details, which can include photograph(s)/tables and/or details within the text to be published in the “Molecular Biology Reports” Journal.

**References**

1. Venkatachalam P, Priyanka N, Manikandan K, Ganeshbabu I, Indiraarulselvi P, Geetha N, Muralikrishna K, Bhattacharya RC, Tiwari M, Sharma N, Sahi SV (2017) Enhanced plant growth promoting role of phycocolloids coated zinc oxide nanoparticles with P supplementation in cotton (Gossypium hirsutum L.). Plant Physiol Biochem 110:118–127. https://doi.org/10.1016/j.plaphy.2016.09.004

2. Wang JA, Zhang FS, Li CJ (2001) Growth of tomato and green pepper under zinc deficiency. Chi J Soil Sci 32:177–179

3. Oktem AG (2019) Effects of different zinc levels on grain yield of a full-sib family of an outbreeding species. Genet Res 93:343–349. https://doi.org/10.1017/S00122-008-0950-9

4. Eken M (2007) Farklı Biber (Capsicum annuum L.) Tiplerinde Çinko (Zn) Etkinliğinin Belirlenmesi. Dissertation, Çukurova Üniversitesi

5. Macnair MR (2007) A quantitative trait loci analysis of zinc uptake and distribution in barley (Hordeum vulgare). New Phytol 174:580–590. https://doi.org/10.1111/j.1469-8137.2007.02036.x

6. Filatov V, Dowdle J, Smirnoff N, Ford-Lloyd B, Newbury HJ, Dang YP, Edwards DG, Dalal RC, Tiller KG (2007) Quantitative trait loci for physical and chemical components of common bean. Crop Sci 43:1029–1035. https://doi.org/10.2135/cropsci2003.1029

7. May F, McVey TJ, McVey KH, Magedanz T, Cakmak I, Waller EA (2011) Quantitative trait loci analysis of Mn and Zn accumulation in barley (Hordeum vulgare). Plant Soil 348:309–322. https://doi.org/10.1007/s11104-010-0603-7

8. Liao L, Li H, Jiang J, Shao D, Xie X, Li Z, Sui X, Zeng X, Chen X, Li X, Fan Y (2013) Identification of quantitative trait loci for zinc status in wheat. Theor Appl Genet 126:2141–2149. https://doi.org/10.1007/s00122-013-2256-6

9. Shams M, Yildirim E, Arslan E, Agar G (2020) Salinity induced alteration in DNA methylation pattern, enzyme activity, nutrient uptake and H2O2 content in pepper (Capsicum annuum L.) cultivars. Acta Physiol Plant 42:1–12. https://doi.org/10.1007/s11738-020-03053-9

10. Williams JG, Kubelek AR, Livaj KV, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535. https://doi.org/10.1093/nar/18.22.6531

11. Van Ooijen JW (2001) Multipoint maximum likelihood mapping in a full-sib family of an outbreeding species. Theor Appl Genet 103:455–461. https://doi.org/10.1007/s001220100570

12. Wu F, Eannetta NT, Xu Y, Tanksley SD (2009) A detailed synteny map of the eggplant genome based on conserved ortholog set II (COSII) markers. Theor Appl Genet 118:927–935. https://doi.org/10.1007/s00122-008-0950-9

13. Williams JG, Kubelik AR, Livak KV, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535. https://doi.org/10.1093/nar/18.22.6531

14. Williams JG, Kubelek AR, Livaj KV, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535. https://doi.org/10.1093/nar/18.22.6531

15. Van Ooijen JW (2001) Multipoint maximum likelihood mapping in a full-sib family of an outbreeding species. Theor Appl Genet 103:455–461. https://doi.org/10.1007/s001220100570

16. Van Ooijen JW, Boer MP, Jansen RC, Maliepaard CA (2000) MapQTL 4.0: software for the calculation of QTL positions on genetic maps (user manual). Wageningen Plant Research International. https://research.wur.nl/en/publications/mapqtl-4-0-software-for-the-calculation-of-qtl-positions-on-genet. Accessed 26 May 2021

17. Dang YP, Edwards DG, Dalal RC, Tiller KG (1993) Identification of an index tissue to predict zinc status of wheat. Plant Soil 154:161–167. https://doi.org/10.1007/BF00012521

18. Huang L, Ye Z, Bell RW (1996) The importance of sampling immature leaves for the diagnosis of boron deficiency in oilseed rape (Brassica napus cv. Eureka). Plant Soil 183:187–198. https://doi.org/10.1007/BF00011434

19. Guzmán-Maldonado SH, Martínez O, Acosta-Gallegos JA, Guevara-Lara F, Paredes-López O (2003) Putative quantitative trait loci for physical and chemical components of common bean. Crop Sci 43:1029–1035. https://doi.org/10.2135/cropsci2003.1029

20. Vreugdenhil D, Aarts MGM, Koornneef M, Nellissen H, Ernst WO (2004) Natural variation and QTL analysis for cationic mineral content in seeds of Arabidopsis thaliana. Plant Cell Environ 27:828–839. https://doi.org/10.1111/j.1365-3040.2004.01189.x

21. Filatov V, Dowdle J, Smirnoff N, Ford-Lloyd B, Newbury HJ, Dang YP, Edwards DG, Dalal RC, Tiller KG (2007) A quantitative trait loci analysis of zinc hyperaccumulation in Arabidopsis halleri. New Phytol 174:580–590. https://doi.org/10.1111/j.1469-8137.2007.02036.x

22. Loneragan PF (2001) Genetic characterisation and QTL mapping of Zn nutrition in barley (Hordeum vulgare). Dissertation, University of Adelaide

23. Sadeghzadeh B, Rengel Z, Li C (2008) Mapping of chromosome regions associated with seed zinc accumulation in barley. Dissertation, University of Western Australia

24. Peleg Z, Cakmak I, Ozturk L et al (2009) Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat × wild emmer wheat RIL population. Theor Appl Genet 119:353–369. https://doi.org/10.1007/s00122-009-1044-z

25. Genc Y, Verbyla AP, Torun AA, Cakmak I, Willsmore K, Wallwork H, McDonald GK (2009) Quantitative trait loci analysis of...
zinc efficiency and grain zinc concentration in wheat using whole genome average interval mapping. Plant Soil 314:49–66. https://doi.org/10.1007/s11104-008-9704-3

26. Wissuwa M, Ismail AM, Yanagihara S (2006) Effects of zinc deficiency on rice growth and genetic factors contributing to tolerance. Plant Physiol 142:731–741. https://doi.org/10.1104/pp.106.085225

27. El-Bendary AA, El-Fouly MM, Rakha FA, Omar AA, Abou-Youssef AY (1993) Mode of inheritance of zinc accumulation in maize. J Plant Nutr 16:2043–2053. https://doi.org/10.1080/01904169309364673

28. Hartwig EE, Jones WF, Kilen TC (1991) Identification and inheritance of inefficient zinc absorption in soybean. Crop Sci 31:61–63. https://doi.org/10.2135/cropsci1991.0011183X003100010015x

29. Genc Y, Shepherd KW, McDonald GK, Graham RD, Leon J (2003) Inheritance of tolerance to zinc deficiency in barley. Plant Breed 122:283–284. https://doi.org/10.1046/j.1439-0523.2003.00845.x

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.