Development of Molecular Markers Linked To QTL/Genes Controlling ZN Efficiency

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Abstract

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.). We constructed a genetic map using an F_2 populations derived from *C. annuum* L. (Alata 21A) X *C. frutescens* L. (PI 281420) cross. The QTLs for Zn efficiency were mapped using $F_{2:3}$ population. A genetic map with 929,6 cM in length and 12 linkage groups were obtained using 62 markers (31 SRAP, 19 SSR and 11 RAPD). 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. The molecular markers are expected to aid selection and expedite breeding peppers resistant to Zn deficiency in soils low for available Zn contents.

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. QTL (Quantitative Trait Locus) 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 Method

Plant materials

The mapping population was derived from a cross between *C. annuum* L. (Alata 21A) and *C. frutenscens* L. (PI 281420) as parents. The F_1 plant was selfed to produce F_2 population (138 plants), and F_3 families were created by selfing F_2 plants. Initially, 455 F_2 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 F_3 seeds were harvested from these plants. The leaf samples were collected for DNA extraction from the F_2 plants during the period when they showed healthy growth under the normal fertilization regime. The 138 genotypes of the 455 F_2 plants were selected for use in mapping. For this purpose, among 455 F_2 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 $F_{2:3}$ family means. The experiment was conducted in a greenhouse and arranged in a completely randomized design with three replications. Each replication consisted of 4 plants of the parents and F_1, and each F_3 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 $(NO_3)_2.4H_2O$, 100 mg kg^{-1} of KH_2PO_4 (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 $ZnSO_4.7H_2O$] 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, F_1, F_2, and F_3 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 F_2 plants, and the parents, using the modified CTAB method as reported by Shams [11].

SSR analysis

The SSR markers that have the highest polymorphism were first screened from the co-dominant marker list that formed the *C. frutescens* x *C. annuum* integrated map. The 138 F_2 individuals were genotyped by markers (corresponding to 12 chromosomes) and 24 markers that had the highest polymorphism were selected for mapping by SRAP and RAPD techniques.

According to sequences and map information was provided on Solgenomics.net [12], sixty primer pairs were tested with parents and among them, 24 primers (Online Resource 1) with the highest polymorphism were selected, and 138 plants of F_2 populations were evaluated.

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 F_2 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].

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 F_2 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 hours. 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 "Krukal 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 x C. frutescens*.

Table 1
Distribution of markers on linkage groups developed from *C. annum x C. frutescens* F_2 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

QTL mapping

QTLs were mapped in the MapQTL.6 software after the LGs was created by JoinMap 4.1 software. The quantitative data obtained from replicated $F_{2:3}$ 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.

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 $F_{2:3}$ populations was analyzed by the MapQTL software. The four QTLs were mapped on LGs 1, 8, 10 and X. The most important markers for this character were EM8ME7.270, OPAH2.290, EM14ME1.480, and EM5ME13.350 (Table 2 and Fig. 1.). The QTLs explained 15.4% of the phenotypic variance of the trait for Zn deficiency symptoms.

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

Trait Number	Trait name	QTL Symbol	Chr	Marker[a]	QTL Position (cM)	R^{2b}	Direction[a]
1	Scores of Zn deficiency symptoms in the F3 population	*f3scor1.1*	1	EM8ME7.270	0.00	2.3	PI 281420
		f3scorx.1	X	OPAH2.290	0.00	2.0	PI 281420
		f3scor8.1	8	EM14ME1.480	73.02	7.9	
		f3scor10.1	10	EM5ME13.350	31.89	3.2	PI 281420
							PI 281420
2	Zn effectiveness in terms of total dry matter weight	*tdmznef3.1*	3	EM6ME6.260	83.67	4.7	PI 281420
		tdmznef8.1	8	EM14ME1.480	73.02	6.9	PI 281420
		tdmznef11.1	11	OP108.370	59.53	6.4	PI 281420
		tdmznefx.1	X	EM11ME8.380	50.04	5.1	PI 281420
3	Zn effectiveness in terms of plant length	*plhtznef3.1*	3	EM6ME6.260	83.68	6.2	PI 281420
		plhtznefx.1	X	EM11ME8.380	50.04	9.2	PI 281420
		plhtznef5.1	5	OPAH2.290	0.00	2.5	PI 281420
		plhtznef6.1	6	OPAC10.330	33.78	3.2	PI 281420
		plhtznef8.1	8	At1G14810	95.53	5.6	PI 281420
		plhtznef9.1	9	GPMS171	41.41	6.0	-
		plhtznef11.1	11	OP108.370	59.53	6.7	-
							PI 281420
4	Zn effectiveness in terms of Zn concentration in total dry matter	*znconef3.1*	3	BM59622	0.00	5.8	-
		znconef6.1	6	C2At1g44760	0.00	4.0	-
		znconef8.1	8	GP20095	47.79	5.1	PI 281420
		znconef9.1	9	EM5ME11.280	0.00	5.1	PI 281420
		znconef9.2	9	GPMS171	41.41	5.0	-
		znconefXXX..1	XXX	OP108.210	0.00	3.2	Alata 21A

[a] Indicates the parent which contributes to the increase in the numeric value of the trait

Trait Number	Trait name	QTL Symbol	Chr	Marker[a]	QTL Position (cM)	R^{2b}	Direction[a]
5	Zn effectiveness in terms of leaf dry matter weight	*ldmwef3.1*	3	EM6ME6.260	83.68	4.5	PI 281420
		ldmwef8.1	8	EM14ME1.480	73.02	5.0	PI 281420
		ldmwef11.1	11	OP108.370	59.53	6.3	PI 281420
6	Scores of Zn deficiency symptoms in the F2 population	*f2scor8.1*	8	GP20095	47.79	6.8	-
		f2scor8.2	8	HPMS1155	31.88	6.0	-
		f2scor10.1	10	OPB01.490	70.34	5.1	PI 281420
7	Zn effectiveness in terms of Zn content in leaves	*lzncntef6.1*	6	OPAC10.330	33.78	3.0	PI 281420
		lzncntef8.1	8	HPMS1155	31.88	5.6	-
		lzncntef11.1	11	OP108.370	59.53	6.2	PI 281420
8	Zn effectiveness in terms of zn content in dry matter	*tdmzncntx.1*	X	OPAH2.290	0.00	5.8	PI 281420
		tdmzncntx.2	X	EM7ME6.200	88.54	3.8	PI 281420
		tdmzncnt8.1	8	EM14ME1.480	73.02	9.9	PI 281420
		tdmzncnt8.2	8	GP20095	47.79	4.1	PI 281420
		tdmzncnt9.1	9	OPI03.350	114.6	4.3	-
		tdmzncnt11.1	11	EM5ME11.220	21.31	5.3	PI 281420
							-
9	Zn effectiveness in terms of Root / Shoot Ratio	*rsref1.1*	1	CAEMS060	40.66	3.4	-
		rsref5.1	5	EM3ME10.290	0.00	2.3	PI 281420
		rsref6.1	6	EM8ME7.190	16.41	5.7	PI 281420
		rsref10.1	10	EM5ME13.260	24.38	3.5	PI 281420
		rsref11.1	11	GP20117	0.00	6.0	PI 281420
							-

[a] Indicates the parent which contributes to the increase in the numeric value of the trait

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 F_3 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 F_1 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, F_1 and F_3 plants regarding to their response to Zn deficiency and at least 4 QTLs on LGs 3, 8, 11, and X were identified.

Zn efficiency measured by plant height

The effect of Zn on plant height was measured using F_3 families grown with or without Zn (Online Resource 2). Plant height showed a wide variation and a transgressive segregation was observed. The effect of Zn deficiency to plant height was mapped on LGs 3, 5, 6, 8, 9, 11 and X. The locations of 4 QTLs on LGs 3, 8, 11 and X were common to the QTLs of the Zn efficiency for total dry matter weight (Table 2 and Fig. 1).

Zn efficiency for Zn concentration in total dry matter

The critical Zn concentration in tissues can vary depending on the plant species, type, plant age, plant part, and environment. All shoots, roots, young leaves, and grains can be used for the determination of Zn content. The most appropriate sample taken for plant nutrient status is leaves [17–18]. Based on the results, even though Alata 21A was the tolerant parent to Zn deficiency, its total dry matter weight was decreased under Zn deficiency. However, total dry matter weight of F_1 plant was higher than the parents and also dry matter weight of the F_3 plant was higher than F_1 plants and parents (Online Resource 2). Alata 21A grew faster than the other parent, and the increase in the number of leaves and area together with the increase in the roots causes to a dilution in Zn concentration in the plant and it can cause to a decrease in the Zn concentration in the total dry matter. However, the PI 281420 is a slow-growing genotype and it helps to provide more stable Zn accumulation within its organs. On the other hand, F_1 plants were able to achieve a value above the average of two parents by providing both rapid development and Zn accumulation in the unit area, hence they can inherit the characteristics of both parents. The F_3 families showed transgressive segregation due to inheriting the characteristics of both parents. Similarly, Sadeghzadeh et al [19] found that Zn concentration in seeds is a multigenic character. In this study, the Zn concentration values (Online Resource 2) in the root, stem, and leaves of the F_2 and F_3 population were used for QTL analysis, and 6 QTLs were mapped on LGs 3, 6, 8, 9 and XXX (Table 2 and Fig. 1). The two QTLs belonging to the Zn efficiency for total dry matter and plant height were located on the same position on LGs 3 and 8. For the Zn concentration of total dry matter, five QTLs explained 28.2% phenotypic variance of this trait (Table 2 and Fig. 1).

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 F_2 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 F_2 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 F_2, two on the LG8, and one on LG10. However, an additional QTL was determined for the Zn deficiency symptom scores of the F_3 population (Online Resource 2). Furthermore, it was estimated that number of genes governing Zn deficiency symptom in the F_2 population is about 1.61 and 1.54 [20–21], indicating two genes control the trait. 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 F_2 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 F_2. The three QTLs explained 14.8% variance of leaf dry matter (Supplement 2 G 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 F_1 plants showed a higher value than both parents, indicative of a heterosis or epistatic effect. Furthermore, as for Zn content in stem and root, F_1 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 Zn 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 F_3 population. The F_1 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 [22–24]. The QTL effective on increasing the Zn content in seed of barely was identified in the short arm of the 2H chromosome [25]. On the other hand, 2 QTLs related to Zn concentration and content in Barely seeds were mapped on the short and long arm of the 2H chromosome [26]. 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 [27] 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 [28] 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 x 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 [29]. In this study, according to the scoring of Zn deficiency symptom, 3 QTL in the F_2 population and 4 QTLs in the F_3 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 [30] who reported that 4 genes control Zn content of leaves in maize. Furthermore, Hartwig et al [31] demonstrated that 3 genes control Zn content in leaf of F_3 population from sensitive and tolerant parents in soybean. On the other hand, Genc et al [32] found that only a non-dominant gene controls Zn activity in F_2 and F_3 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 F_2, and F_3 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

Declarations

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Contributions

HP and NM designed the experiment and drafted the manuscript and did molecular experiments. DB and CB carried out soil Zn testing. MKS and SE did data analysis and edited the manuscript.

Conflicts 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.

Ethical approval

The present manuscript has not been published anywhere else in the online or printed version. The author and corresponding author have no restrictions on data availability sharing, literature and all the software were collected from public sources. The manuscript data, illustrations or figures, and any other materials do not infringe any existing copyright or other rights of anyone.

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Figures

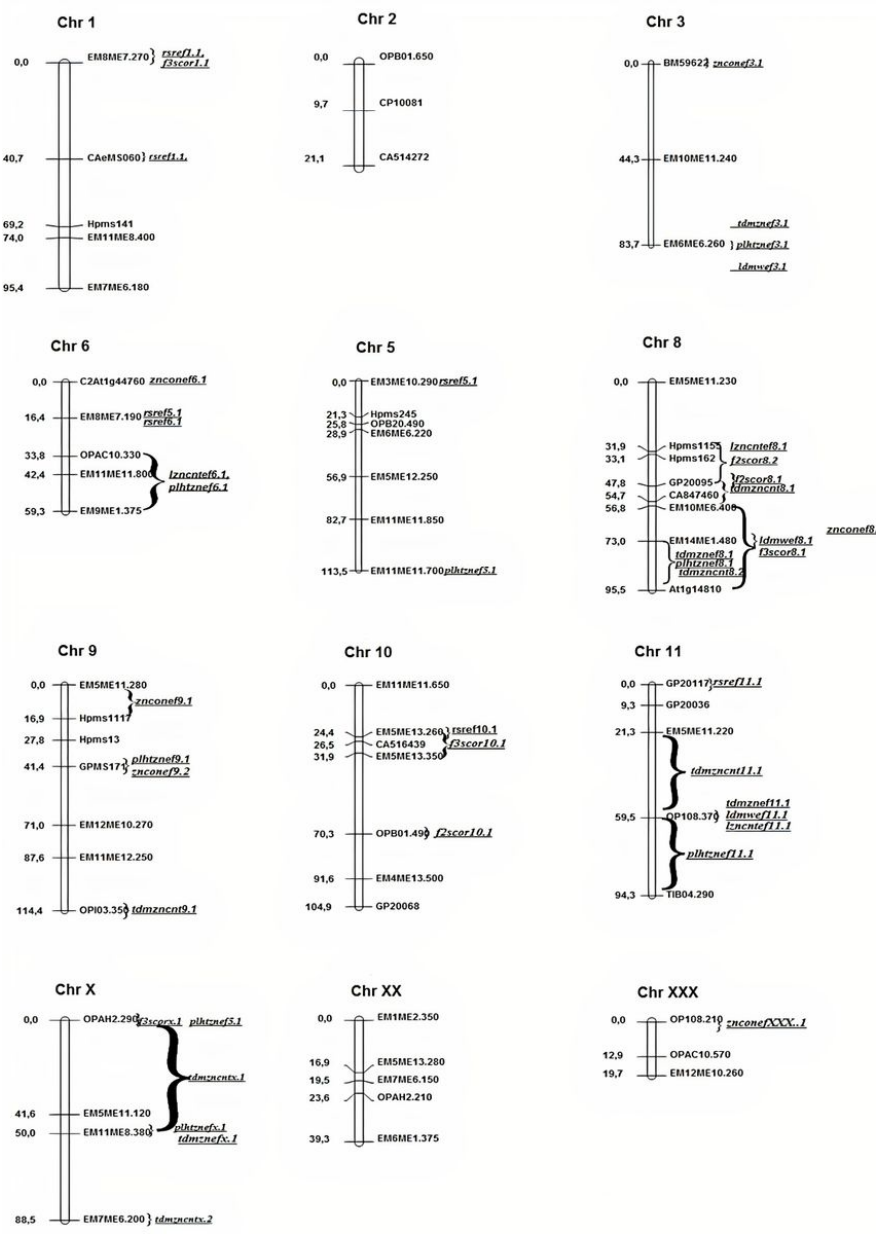

Figure 1

Distribution of SSR, SRAP and RAPD markers and Zn efficiency related QTLs on 12 linkage groups of pepper

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