Uluslararası Tarım Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Agricultural Research 2021, Vol. 5 (1), 91-102
https://doi.org/10.29329/ijiaar.2021.339.7
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Original article

Grouping Some Paddy Cultivars in Terms of Zinc Nutrition Capabilities and Identification of the Best Zinc Nutrition Traits of These Cultivars

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Abstract

The present study was conducted to group some paddy cultivars in terms of their zinc nutritional capabilities and to identify the best zinc nutrition traits of these cultivars. Present experiments were conducted with 5 different paddy cultivars (Oryza sativa L. cv Biga İncisi, Osmancık 97, Hamzadere, Ronaldo, Edirne). In the experiment, a complete nutrient solution containing 0 and 2.0 µM Zn in the form of zinc sulphate heptahydrate was applied to lime-free (0 % CaCO3) and lime-added (4 % CaCO3) quartz sand media. Experiments were conducted in 5×2×2 factorial design with 3 replications. At the end of the experiments, zinc nutrition traits of paddy cultivars were determined. Both in lime-free and lime-added sand media, Biga İncisi paddy cultivar was identified as tolerant to zinc deficiency. Ronaldo paddy cultivar was identified as the most sensitive cultivar to zinc deficiency. It was observed that in terms of investigated 15 zinc nutrition traits, 5 different paddy cultivars grown under zinc deficiency (Zn0) conditions were gathered under 2 main groups. Biga İncisi and Edirne cultivars constituted the first group; Osmancık-97 and Hamzadere cultivars constituted the second group. On the other hand, Ronaldo cultivar was found to be closer to the second group including Osmancık-97 and Hamzadere cultivars. The closest paddy cultivars grown under zinc deficiency conditions in terms of investigated traits were identified as Biga İncisi and Edirne cultivars; on the other hand, the furthest cultivars were identified as Biga İncisi and Osmancık-97 cultivars. For Osmancık-97 and Biga İncisi paddy cultivars, SPAD readings in lime-added sand media were identified as the best zinc nutrition traits. For Edirne cultivar, zinc ratio transported to shoot and shoot relative dry matter content in lime-added media were identified as the best zinc nutrition traits. For Hamzadere cultivar, relative chlorophyll content in lime-free media was identified as the best zinc nutrition.

Keywords: Paddy cultivar, Zinc nutrition, Sensitive cultivar, Resistant cultivar.

Received: 16 March 2021 * **Accepted:** 26 March 2021 * **DOI:** https://doi.org/10.29329/ijiaar.2021.339.7

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INTRODUCTION

Zinc is an essential micro element for all living organisms. It plays an important role in various biological processes and cellular metabolisms including protein synthesis, sugar conversion into starch, carbohydrate and auxin metabolism, pollen formation, resistance mechanisms against pathogen infections and antioxidative protection (Alloway, 2004; Clemens, 2006; Broadley et al., 2007).

Zinc is a critical component of enzymes and proteins (Marschner, 1995) and play a role in binding and direction between the enzymes and substrate bonds (Çakmak, 2000). Zinc activates especially the carbonic anhydrase and superoxide dismutase enzymes; these enzymes are localized in chloroplasts (Jakobsan et al., 1975; Marschner, 1995). Zinc also has direct contributions to RNA synthesis and termination of RNA synthesis and protein production accordingly were reported under zinc deficiency (Price, 1962).

Inundation-induced zinc deficiency was reported in paddy fields. Such a case probably resulted from bicarbonate (HCO_3^-) of water. Regardless of pH, zinc uptake of paddy plant is always at low levels under anaerobic conditions. Reduction in zinc availability under relevant conditions was mostly resulted from zinc-sulphur precipitation or probably from formation of organic zinc complexes (Ponnnamperuma, 1972). Cellulose supplementations to submerged soils further aggravate zinc deficiency. Organic matter supplementations increase reduction conditions in soils and result in formation of greater quantity of Fe^{+2}, such a case then inhibits zinc uptake. With organic matter degradation, water HCO_3^- concentration of paddy basins increase and then zinc transport from roots to upper parts of paddy fields is negatively influenced. Zinc deficiency is generally encountered in paddy fields and widespread based on high pH, low available zinc level and high organic carbon contents. It was determined that easily decomposed organic matter aggravated zinc deficiency in paddy plant. High HCO_3^- concentrations released through decomposition of organic matter immobilize zinc around the paddy roots and inhibit zinc transport to shoots. The organic acids produced through anaerobic decomposition of organic matter and microbial metabolism products also contribute to relevant zinc deficiency problems. It was also reported that the soils with high clay and organic matter content had greater adsorption capacity and zinc-binding energy than the sandy soils poor in organic matter (Brohi et al., 1994).

Yoshida and Tanaka (1969) indicated that zinc deficiency in paddy plants is generally encountered 2-3 weeks after transplantation of paddy seedlings into growing environment. Various symptoms are encountered in plants in case of zinc deficiency. Depending on severity of zinc deficiency, brown spots or lines covering almost the entire leaf surface are encountered in old leaves, plants exhibit dwarf development, ripening is delayed and significant decreases are experienced in yields. Additionally, in case of severe zinc deficient, plant die outs were also reported.

Yoshida et al. (1973) indicated varied ability of some paddy cultivars to grow in zinc-deficient soils. Also, weak growth and less tillering were reported in some paddy varieties sensitive to zinc deficiency.

Bowen (1986) reported that paddy (*Oryza sativa* L.) cultivars exhibited significant differences in terms of sensitivity to zinc (Zn) deficiency.

This study was conducted to group paddy cultivars in terms of zinc nutrition capability and to determine the best zinc nutrition characteristics of these cultivars.

MATERIALS and METHODS

Experiments were conducted in sand media-filled pots in 2018-2019 growing season under controlled conditions of a glass-covered greenhouse in Agricultural Faculty of Ondokuz Mayıs University.

Paddy Genotypes Used in Greenhouse Experiments

Paddy seeds used in greenhouse experiments were supplied from Black Sea Agricultural Research Institute, Edirne Agricultural Research Institute and Ondokuz Mayıs University Agricultural Faculty Field Crops Department. Five different paddy cultivars were used to determine zinc nutrition capability. These cultivars are: Biga İncisi, Osmancık-97, Hamzadere, Ronaldo and Edirne paddy cultivars (Table 1).

Table 1. Some characteristics of paddy cultivars

Paddy cultivars	Hybridization	Vegetation duration (day)	Plant height (cm)	Thousand-grain weight (g)
Biga İncisi	*Baldo x Koral*	125-130	115-120	38-40
Osmancık-97	*Rocca x Europa*	95-100	130-135	33-34
Hamzadere	*Demir x 83013-TR631-4-1-2*	130	95	37-38
Ronaldo	-	135-140	75-80	32-33
Edirne	*Baldo x Calendal*	125-130	105-110	38-39

Experiments

Paddy seeds were kept in 5.0 % (v/v) sodium hypochlorite solution for 15 minutes for seed sterilization. Then, seeds were washed through deionized water and germinated in moist fabric bags. Germinated seeds were transferred to perlite-filled containers (40 x 25 x 5 cm dimensions) and grown there for 10 days to get paddy seedlings. Seedlings of 5 different paddy cultivars were transplanted into plastic pots (12 x 12 cm) filled with 1 kg quartz sand (0 and 4 % $CaCO_3$, lime-free and lime-added) as to have 10 seedlings in each pot. At each lime dose, following plant nutrient solutions (Zhang et al., 1998) containing 0 and 2 µM Zn doses were applied:

500 µM NH_4NO_3; 60 µM $NH_4H_2PO_4$; 230 µM K_2SO_4; 210 µM $CaCl_2$; 160 µM $MgSO_4 \cdot 7H_2O$; 2.5µM $MnCl_2$; 0.75 µM $(NH_4)_6Mo_7O_{24}$; 3.2 µM H_3BO_3; 0.1 µM $CuSO_4$; 45 µM Fe (Fe-EDDHA)

Nutrient solutions were applied to 5 different paddy cultivars at equal quantities as to have 3 cm water head over the sand in experimental pots. Nutrient solution pH was adjusted at 5.5 with the use of dilute HCl or KOH solution. Experiments were conducted in factorial experimental design with two different lime doses, 2 different zinc doses and 5 different paddy cultivars (2 × 2 × 5). Experiments were conducted in 3 replicates and lasted for 50 days.

Phenological observations were made and fresh leaf samples were taken about a week ahead of the harvest. Chlorophyll and carotenoid analyses were conducted on these leaf samples. Again, a week ahead of the harvest, SPAD measurements were conducted on fresh leaves of the paddy cultivars.

At the end of the experiments, roots and above-ground parts of paddy plants were harvested and fresh weights were determined with a precise balance. Paddy cultivars were separated into roots and above-ground parts and dried in an oven at 65 ºC. Then, dry root and shoot weights were determined. Roots and shoots were ground in a grinder with stainless steel blades and made ready for analyses.

Plant Analysis

Root and shoot total iron and zinc contents were determined with the use of atomic absorption spectrophotometer (AAS) device in accordance with Kacar and İnal (2008).

In fresh leaf samples, chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid absorbance readings were performed in a spectrophotometer device as specified in Arnon (1949) and Witham et al. (1971) and calculations were made with the use of the following equations:

Chlorophyll-a (mg g fresh matter $^{-1}$) = $[12.70*A_{663} – 2.69*A_{645}] * V / (1000*W)$

Chlorophyll-b (mg g fresh matter $^{-1}$) = $[22.90*A_{645} – 4.68*A_{663}] * V / (1000*W)$

Total chlorophyll (mg g fresh matter $^{-1}$) = $[20.2*A_{645} + 8.02*A_{663}] * V / (1000*W)$

Carotenoid (mg g fresh matter $^{-1}$) = $A_{480}*V / (250*W)$

A_{663} = Absorbance at 663 nm

A_{645} = Absorbance at 645 nm

A_{480} = Absorbance at 480 nm

V = Final volume, mL

W = Sample weight, g fresh matter

Portable SPAD meter (Konica Minolta SPAD-502 Plus) device was used to get SPAD readings. Active iron of dry leaf samples was determined with the use of AAS device (Oserkowsky, 1933).

Root and shoot zinc uptakes were calculated as follows:

Root or shoot zinc uptake (µg Zn / pot) = Rot or shoot zinc content (ppm) × root or shoot dry weight (g)

The zinc ratio transported to shoot was calculated as follows:

The ratio of zinc transported to plant shoot (%) = (A / B) × 100

A = Shoot zinc uptake, µg Zn / pot

B = Shoot + root zinc uptake, µg Zn / pot

The ratio of zinc remained in root was calculated as follows:

The ratio of zinc remained in root (%) = (C / D) × 100

C = Root zinc uptake, µg Zn / pot

D = Shoot + root zinc uptake, µg Zn / pot

Zinc deficiency tolerance index of paddy cultivars (relative values) was calculated as follows:

Zinc deficiency tolerance index values, % = (A / B) × 100

A: Values for relevant traits of the cultivars grown in non-zinc containing nutrient solution

B: Values for relevant traits of the cultivars grown in 2 µM Zn containing nutrient solution

Table 2. Visual assessment scale for zinc deficiency in paddy plant (IRRI, 2002)

Severity of deficiency	Symptoms encountered in plant
1	Development and tillering are normal, healthy
2	Development and tillering are normal, slight green color loss in basal sections of the leaves
3	Slight dwarf, low tillering, brown or yellow color in basal sections of some leaves
5	Development and tillering are seriously inhibited, almost half of the leaves are brown or yellow
7	Development and tillering are terminated, majority of the leaves are brown or yellow
9	Almost all plants die

Statistical Analyses

Cluster or similarity test was conducted to determine closeness and distance of 5 different paddy cultivars to each other in terms of zinc nutrition index. Cluster analysis was conducted in accordance with Ward Method with the use of JMP.5.0 statistical software. Biplot analysis was conducted for classification of paddy cultivars based on zinc nutrition index, variation of paddy cultivars in terms of this index and to determine the best traits of each cultivar.

RESULTS and DISCUSSION

Comparison of Paddy Cultivars Grown under Zinc Deficiency (Zn0) Conditions

The Zn deficiency tolerance values (relative values) for 15 traits investigated for grouping, closeness-distance status and the best traits of paddy cultivars grown under zinc deficiency (Zn0) conditions are provided in Table 3.

Table 3. Zn deficiency tolerance values (relative values) for 15 traits investigated for grouping, closeness-distance status and the best traits of paddy cultivars grown under zinc deficiency (Zn0) conditions

Cultivars	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
Biga incisi	96.95	91.96	110.63	80.25	81.13	95.08	74.42	94.89	54.76	83.11	2	67.4	64.9	32.5	35
Osmancık-97	70.27	74.27	65.24	65	85.19	78.91	80.15	74.81	85.64	82.82	2	64.4	57	35.5	43
Hamzadere	73.31	78.45	75.88	80.25	97.36	97.34	91.6	93.06	96.33	75.82	2	62	60	37.9	39.9
Ronaldo	71.62	72.02	55.31	56.17	89.24	107.37	87.78	100.36	94.45	84.77	3	61.2	53.2	38.7	46.7
Edirne	91.21	104.18	91.48	80.25	91.61	86.67	87.78	83.94	77.86	82.72	2	70.9	60.3	29	39.6

Information on the characteristics of the roman numbers in the table is given below.

Abbreviations: (K0: lime-free sand media; K1: lime-added sand media)

K0 relative weight of shoot dry matter (I); K1 relative amount of shoot dry matter (II); K0 Relative zinc uptake in shoot (III); K1 Relative zinc uptake in shoot (IV); K0 relative total chlorophyll content (V); K1 relative total chlorophyll content = (VI); K0 relative carotenoid content (VII); K1 relative carotenoid content (VIII); K0 relative SPAD reading value (IX); K1 relative SPAD reading (X); K0 Zn deficiency symptom scale (XI); K0 The ratio of zinc carried to the shoot (XII); K1 ratio of zinc carried to the shoot (XIII); K0 ratio of zinc remaining in the root (XIV); K1 ratio of zinc remaining in the root (XV)

[*] Relative value is calculated as % = [value determined at Zn0 level / value determined at sufficient zinc (Zn2) level] × 100

The values indicating closeness and distance levels of paddy cultivars grown under zinc deficiency (Zn0) conditions to each other are provided in Table 4.

Table 4. Closeness and distance levels of paddy cultivars grown under zinc deficiency conditions to each other

Level	Number of groups	Similarity level	Distance level	Linkage between the groups		New groups	Number of cultivars in each group
1	4	70.9308	15.8224	1	5	1	2
2	3	61.0641	21.1929	2	3	2	2
3	2	51.7248	26.2764	2	4	2	3
4	1	-4.1851	56.7083	1	2	1	5

The dendrogram for grouping of paddy cultivars grown under zinc deficiency (Zn0) conditions is presented in Figure 1.

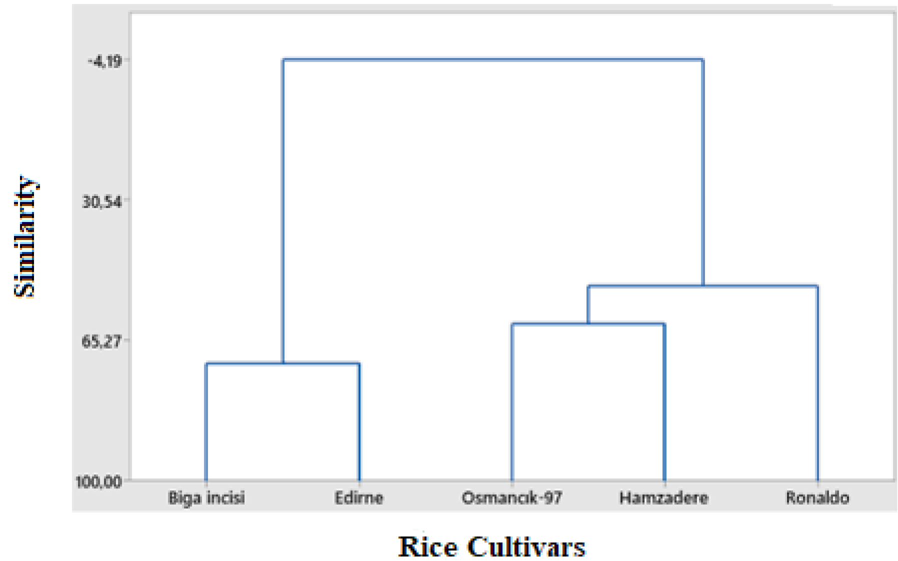

Figure 1. The dendrogram generated with the use of Ward method, explaining closeness and distance of paddy cultivars to each other based on relative values [*] investigate traits determined under zinc deficiency conditions

As can be inferred from Table 3 and Figure 1, in terms of investigated 15 traits, 5 different paddy cultivars grown under zinc deficiency (Zn0) conditions were gathered under 2 main groups. While Biga İncisi and Edirne cultivars were placed into the first group, Osmancık-97 and Hamzadere cultivars were placed into the second group. On the other hand, Ronaldo cultivar were found to be closer to the second group including Osmancık-97 and Hamzadere cultivars. The closest paddy cultivars grown under zinc deficiency conditions in terms of investigated traits were identified as Biga İncisi and Edirne cultivars (level of distance 15.82); on the other hand, the furthest cultivars were identified as Biga İncisi and Osmancık-97 cultivars (level of distance 56.70). Phuke et al. (2017) reported that had highly significant effect of interactions between cultivars and their Zn nutrition capabilities, and also between genotype and environment biplots comparison for grain Zn content showed possibility of simultaneous effective selection for the traits.

Classification of paddy cultivars grown under zinc deficiency (Zn0) conditions based on mean values of investigated 15 traits and variations of cultivars in terms of investigated traits are presented in Figure 2. According to biplot analysis, two principal components (PC1 and PC2) explained 74.7 % of total variation (PC1 explaining 55.2 % and PC2 explaining 19.5 %). As can be inferred from Figure 2, investigated traits and distribution of cultivars varied with the cultivars. Relative SPAD value was identified as the best trait of Osmancık-97 and Biga İncisi cultivars grown in lime-supplemented sand media under zinc deficiency conditions. Relative SPAD value was measured as 82.82 % for Osmancık-97 cultivar and 83.11 % for Biga İncisi cultivar (Table 2); considering these values of Osmancık-97 and Biga İncisi paddy cultivars, they were considered to be resistant to zinc deficiency. Total relative chlorophyll content was identified as the best trait of Hamzadere cultivar grown in lime-free sand media under zinc deficiency conditions. Total relative chlorophyll content was identified as 97.36 % for Hamzadere cultivar grown in lime-free sand media under zinc deficiency conditions; with this value, the cultivar was considered to be resistant to zinc deficiency. Zinc ratio transported to shoot and shoot relative dry matter content were identified as the best traits of Edirne paddy cultivar grown in lime-supplemented (4 % $CaCO_3$) sand media under zinc deficiency (Zn0) conditions; relevant values of the cultivar were respectively identified as 60.3 % and 104.18 % (Table 3). Considering these values, Edirne cultivar was identified as a well-cultivar in lime-added sand media under zinc deficiency conditions.

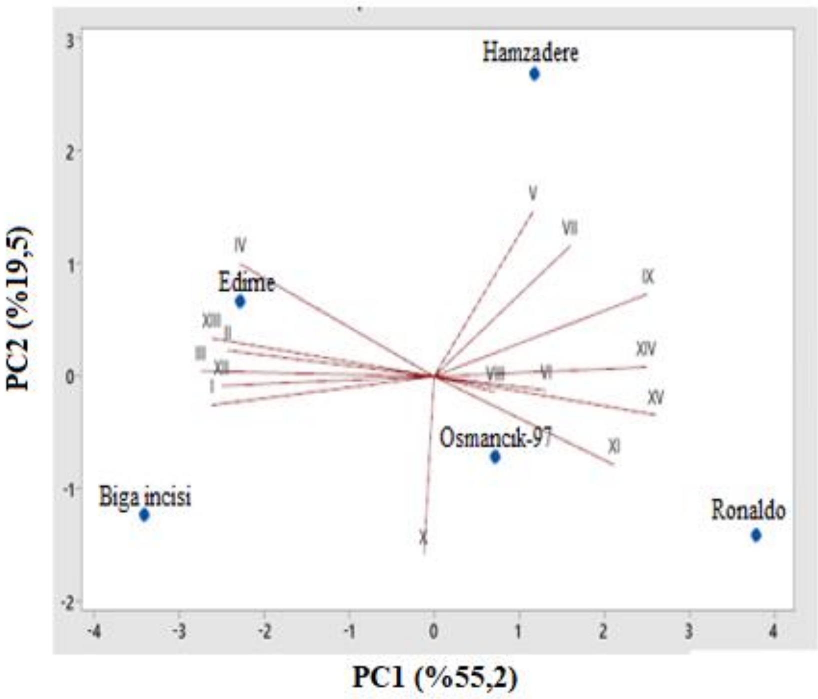

Figure 2. Biplot grouping of paddy cultivars under zinc deficiency conditions based on investigated traits and best traits of the cultivars

Symptom Levels of Paddy Cultivars against Zinc Deficiency and Zinc Deficiency Tolerance Index Values

Symptom levels of paddy cultivars under zinc deficiency conditions against zinc deficiency and zinc deficiency tolerance index values of paddy cultivars grown in lime-free and lime-added sand media are provided in Table 5.

Table 5. Visually diagnosed chlorosis levels of paddy cultivars grown in lime-free and lime-added sand media and zinc deficiency tolerance index values of the cultivars

Paddy cultivars	Lime-free sand media		Lime-added sand media	
	Symptom level (IRRI, 2002)	**Zinc deficiency tolerance index, %**	**Symptom level (IRRI, 2002)**	**Zinc deficiency tolerance index, %**
Biga İncisi	2	97.00	2	91.66
Osmancık-97	2	70.00	2	74.03
Hamzadere	2	73.30	2	78.20
Ronaldo	3	71.60	3	71.79
Edirne	2	91.20	2	83.93

As can be inferred from Table 5, in terms of visual diagnosis of zinc deficiency symptoms, Ronaldo paddy cultivar grown in lime-free and lime-added (4 %) sand media was classified in 3rd level (slight dwarf, low tillering, brown or yellow color in basal sections of some leaves). Zinc deficiency tolerance index of this cultivar in lime-free and lime-added sand media was respectively identified as 71.60 % and 71.79 %. In terms of visual diagnosis of zinc deficiency symptoms, the other cultivars were classified in 2nd level (Development and tillering are normal, slight green color loss in basal sections of the leaves). Zinc deficiency tolerance index of Biga İncisi cultivar in lime-free and lime-added sand media was respectively identified as 97.0 % and 91.66 %. Zinc deficiency tolerance index of Osmancık-97 cultivar in lime-free and lime-added sand media was respectively identified as 70.05 % and 74.03 %. Zinc deficiency tolerance index of Hamzadere cultivar in lime-free and lime-added sand media was respectively identified as 73.30 % and 78.20 %. Zinc deficiency tolerance index of Edirne cultivar in lime-free and lime-added sand media was respectively identified as 91.20 % and 83.93 %. Gao et al. (2005) assessed the variation in zinc efficiency (ZE) of a large Chinese paddy genotype set including some newly-developed aerobic paddy genotypes and reported reduced shoot elongation and whitish brown necrotic spots on leaves 3-4 weeks after the transplantation. Significant differences were observed in zinc deficiency symptoms of 23 different paddy genotypes. The K150, Han297, Yuefu, Xieyou10 and IR26 paddy genotypes exhibited severe zinc deficiency symptoms. On the other hand, Han44, Hongkelaoshuya, Jindao305 and IR8192-31 paddy genotypes did not exhibit a zinc deficiency symptom under non-zinc applied conditions. Under zinc treatments, all paddy genotypes exhibited well growth and development and did not exhibit any zinc deficiency symptoms. Besides, Zn deficiency remarkably reduced both root and shoot dry weight of majority of the genotypes. Shoot-based ZE values varied between 50 – 98 %. Although shoot-based zinc efficiencies exhibited a broader range, they complied with root-based zinc efficiencies. The aerobic paddy genotypes of Hongkelaoshuya, Han44 and lowland genotypes of IR8192-31, Jindao305 were identified as the most tolerant genotypes to Zn deficiency; their ZE values were around 95 %. The aerobic genotype of K150 and lowland genotypes of Yuefu, Xieyou10, IR26 were identified as the most sensitive genotypes to Zn deficiency; their ZE values varied between 50 – 61 %. Additionally, lowland genotypes generally had lower zinc efficiencies than the aerobic genotypes. Zn-efficient genotypes including Hongkelaoshuya, Han44, IR8192-31 were able to preserve similar root biomass with Zn treatments as compared to Zn-deficit conditions. However, non-Zn-efficient genotypes including K150, Han297, Yuefu and Xieyou10 experienced about 40 % reduction in root biomass under Zn-deficit conditions. In a study conducted by Humaira et al. (2015), 4 different paddy cultivars were grown under hydroponic conditions with two different zinc concentrations (0, 1 and 2 µM Zn) and effects of Zn concentrations on plant height, crude protein, chlorophyll-a, chlorophyll-b, total zinc and iron contents were investigated. At 1,0 uM Zn dose, IRRI-6 and Basmati- 2000 paddy cultivars had greater plant heights than JP-05 and Swat-I cultivars. While JP-05 and Basmathi-2000 cultivars had the maximum crude protein contents at 1 uM Zn dose, Swat-1 and IRRI-6 cultivars had the maximum levels at 2 µM Zn dose. A similar case was also observed in chlorophyll-a and chlorophyll-b contents of the paddy cultivars.

CONCLUSION

Both in lime-free and lime-added sand media, Biga İncisi paddy cultivar was identified as tolerant to zinc deficiency. Ronaldo paddy cultivar was identified as the most sensitive cultivar to zinc deficiency. It was observed that in terms of investigated 15 zinc nutrition traits, 5 different paddy cultivars grown under zinc deficiency (Zn0) conditions were gathered under 2 main groups. Biga İncisi and Edirne cultivars constituted the first group; Osmancık-97 and Hamzadere cultivars constituted the second group. On the other hand, Ronaldo cultivar was found to be closer to the second group including Osmancık-97 and Hamzadere cultivars. The closest paddy cultivars grown under zinc deficiency conditions in terms of investigated traits were identified as Biga İncisi and Edirne cultivars; on the other hand, the furthest cultivars were identified as Biga İncisi and Osmancık-97 cultivars.

For Osmancık-97 and Biga İncisi paddy cultivars, SPAD readings in lime-added sand media were identified as the best zinc nutrition traits; for Hamzadere cultivar, relative chlorophyll content in lime-free media was identified as the best zinc nutrition; for Edirne cultivar, zinc ratio transported to shoot and shoot relative dry matter content in lime-added media were identified as the best zinc nutrition traits.

Acknowledgement

We would like to thank all staffs of Agricultural Biotechnology Laboratory of Ondokuz Mayıs University for their kind support during this research.

This paper was derived from the Ph.D. Thesis of Dr. Güney Akınoğlu conducted under the supervision of Prof. Dr. Ahmet Korkmaz.

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