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Application of Zinc Mitigates the Salt-Induced Effects on Growth of Soybean (*Glycine max* L.)

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Abstract: Salinity is a major issue restricting sustainable soybean production in arid and semi-arid regions of the world. Zinc is an important micronutrient which may improve plant growth and development. Therefore, a research was undertaken to clarify the role of zinc on growth of soybean under salt stress. The experiment was conducted using six levels (0, 50, 100, 150, 200 and 250 mM) of NaCl and the plant was also treated with zinc under different salinity levels. This experiment was arranged in a completely randomized design with three replications. Maximum salt stress (250 mM NaCl) caused reduction in plant height by 64.35%, 58.82%, 56.90% and 57.37%, leaf number by 72.68%, 65.31%, 57.57% and 53.41% and leaf area by 84.31%, 76.67%, 70.61%, and 67.96% at 15, 30, 45 and 60 days after treatment, respectively. Whereas, application of Zn to salt-stressed plants elevated plant height by 82.38%, 49.24%, 49.93%, and 43.51%, leaf number by 41.02%, 16.48%, 2.61%, and 11.28% and leaf area by 41.85%, 40.09%, 18.47% and 17.67% at 15, 30, 45 and 60 days after treatment, respectively. These results indicate that zinc plays an important role on growth of salt-stressed soybean. Zn application compensated the deleterious effects of Na^+ and Cl^- ions and led to greater plant growth.

Keywords: Salinity, Growth, Yield, Soybean

1. Introduction

Salinity is one of the major environmental stresses hampering crop growth and development, consequently lowering global crop production. About 1125 million hectares of land worldwide are salt-affected, which represents 6% of world's total area including 20% of cultivated and 33% of the arable land [1, 2]

The impact of salt stress is in the reduction of plant growth which is initially caused by osmotic stress and then followed by ion toxicity [3]. Furthermore, salt accumulation in leaves leading to salt toxicity in the plants. Moreover, In plant cells, the plethora of toxic ions causing damages in membrane systems and organelles, leading to reduced plant growth and abnormal development prior to plant death [4–6]. Salt stress causes changes in gene expression in the majority of plants, accelerating the synthesis of osmoprotectors and osmoregulators [7]. Salinity often leads to increased uptake of Na^+ or decreased uptake of Ca^{2+} and K^+ in leaves which causes nutritional imbalances [8]. Salt stress causing the death of leaves due to scarcity of nutrients, therefore reducing the total photosynthetic leaf area which reduces the supply of photosynthate in plants and ultimately it affects the yields [9].

Zinc is a crucial element for plant growth, as it is involved in various vital cellular functions such as protein, carbohydrate, and carbon metabolism, as well as IAA metabolism and cell division [10–13]. Zn supplementation could potentially assist to combat salt-induced harmful effects [14–17].

Soybean (*Glycine max*) belongs to fabaceae family native to East Asia, widely grown for its edible bean which has numerous uses. Dry soybean contain 36% protein, 19% oil, 35% carbohydrate (17% of which dietary fiber), 5% minerals and several other components including vitamins [18]. According to FAO [19], globally the total annual production of soybean is 260.91 million tons from an area of 102.99 million hectares. Farmers in Bangladesh grow cereal crops on good soil for food security, but they cultivate non-cereal crops on marginal terrain, such as the southern coastal strip. In contrast, around 20% of land in southern coastal strip of

Bangladesh is afflicted by varied degrees of salinity [20]. Soybean cultivation covers a total area of 41440 hectares, yielding 65883 tons of oil every year [19]. There is increasing evidence that salt stress has a major impact on growth and yield of soybean, making it as a salt sensitive crop. However, soybean production may not achieve its goal due to natural disasters, soil salinity and ambient air pollution in Bangladesh. As a result, the current study tried to explore the combined effects of Zn and salinity on different growth stage of soybean (cv. Shohag).

2. Materials and Methods

2.1. Plant Materials

Soybean (cv. Shohag) seeds were obtained from the Bangladesh Agricultural Research Institute (BARI) and used as plant materials.

2.2. Treatments

The experiment used a completely randomized design with three replications. Treatments were performed at six NaCl concentrations (0, 50, 100, 150, 200 and 250 mM) with and without zinc addition.

2.3. Pot preparation and Management Practices

Pot experiment were conducted in the experimental field of Jahangirnagar University's Botanical Garden in Savar, Dhaka. The soil sample was air-dried and pulverized. Before sowing 0.41 gm TSP, 0.5 gm MP was mixed into the soil per pot, and enough water was added to saturate the soil. Then zinc was combined thoroughly with soil at a rate of 10 mg kg^{-1} as $ZnSO_4.7H_2O$. 18 pots were filled with zinc treated soil. The pots were kept under natural sunshine till harvesting. The seeds were surface sterilized by soaking them for three minutes in 0.1% sodium hypochlorite and then washing them three times in distilled water. Seeds of uniform size were directly sown on 4^{th} February 2019. Distilled water was applied in all pots up to the emergence of seedlings. After seedling establishment distilled water in control pots and 12.5 mM NaCl solution were applied in salt treatment. When the first leaf appeared, actual amount of NaCl solution were applied. The salt solution was used until it became mature. Regular watering and weeding were made to ensure equal environmental condition throughout the experimental period.

2.4. Measurement of Growth

Plant height, leaf length, leaf breadth, leaf number and leaf area of soybean cultivar were measured at 15, 30, 45 and 60 days after treatment (DAT).

2.5. Statistical Analysis

Statistical analysis of the collected data was performed using Analysis of Variance (ANOVA). All statistical analysis was performed with the SPSS statistical package (SPSS program 16.00). The data were presented as the means for each treatment. Means were compared using the LSD test at the 5% probability level.

3. Results

3.1. Plant Height

The data on plant height of soybean cultivar recorded at 15, 30, 45 and 60 DAT are presented in the Table 1. Plant height significantly influenced by salinity levels. Compared with control, plant height of soybean treated with 200 mM NaCl decreased by 47.94%, 51.38%, 49.21%, and 52.54% at 15, 30, 45 and 60 DAT, respectively. Compared to plants subjected to 200 mM NaCl, Zn supplementation (200 mM NaCl + Zn) enhanced plant height by 52.02%, 50.16%, 46.75% and 39.95% at 15, 30, 45 and 60 DAT, respectively. Compared with control, heights of plants treated with 250 mM NaCl decreased by 64.35%, 58.82%, 56.90% and 57.37% at 15, 30, 45 and 60 DAT, respectively. Zn supplementation (250 mM NaCl + Zn) increased plant height by 82.38%, 49.24%, 49.93%, and 43.51% at 15, 30, 45, and 60 DAT, respectively, as compared to plants exposed to 250 mM NaCl.

Table 1. *Effects of zinc on plant height (cm) of soybean cv. Shohag at different days after treatment under salinity.*

Treatments	Days after treatment (DAT)			
	15	30	45	60
0 mM NaCl	68.83±2.36 a	82.00±2.64 a	91.80±2.45 a	109.07±5.95 a
50 mM NaCl	58.00±5.57 b (84.27)	74.40±4.05 a (90.73)	78.80±2.52 b (85.84)	83.93±4.86 b (76.95)
50 mM NaCl + Zn	65.67±4.21 ab (95.41)	76.37±5.51 b (93.13)	82.17±8.27 b (89.51)	99.17±5.53 b (90.92)
100 mM NaCl	45.47±3.50 c (66.06)	53.83±9.46 b (65.64)	61.70±7.45 c (67.21)	68.76±7.86 c (63.04)
100 mM NaCl + Zn	65.33±4.51 ab (94.91)	71.00±8.19 b (86.59)	78.20±6.72 b (85.19)	87.17±7.75 bc (79.92)
150 mM NaCl	38.67±2.08 d (56.18)	47.67±4.5 bc (58.13)	51.83±3.17 d (56.46)	58.27±9.53 cd (53.42)
150 mM NaCl + Zn	58.27±11.12 abc (84.66)	63.10±9.85 bc (76.95)	70.43±12.04 bc (76.72)	75.00±4.36 cd (68.76)
200 mM NaCl	35.83±1.93 d (52.06)	39.87±3.35 cd (48.62)	46.63±2.03 de (50.79)	51.77±1.37 de (47.46)
200 mM NaCl + Zn	54.47±10.04 bc (79.65)	59.87±9.27 bc (73.01)	68.43±4.40 bc (74.54)	72.47±3.05 d (66.44)
250 mM NaCl	24.40±4.41 e (35.45)	33.77±4.13 d (41.18)	39.57±4.50 e (43.10)	46.50±4.27 e (42.63)
250 mM NaCl + Zn	44.50±8.32 c (64.65)	50.40±9.18 c (61.46)	59.33±8.33 c (64.63)	66.73±6.60 d (61.18)

* Average value of 9 plants in each treatment.

* Means in a column followed by the same letter do not differ significantly at 5% level and±means standard deviation.

* Values within parenthesis indicate percentage relative to the control.

3.2. Leaf Number

The data on leaf number of soybean cultivar recorded at 15, 30, 45 and 60 DAT are presented in the Table 2. Salinity disturbed seriously the production of leaf, which was depicted in the stiff reduction in leaf number/plant with the increasing levels of salinity. Compared with control, leaf number of plants treated with 200 mM NaCl decreased by 72.68%, 57.14%, 48.11% and 44.81% at 15, 30, 45 and 60 DAT, respectively. Compared to plants subjected to 200 mM NaCl, Zn supplementation (200 mM NaCl + Zn) enhanced leaf number by 90%, 25.71%, 11.65%, 16.24% at 15, 30, 45 and 60 DAT, respectively. Compared with control, leaf number of plants treated with 250 mM NaCl decreased by 72.68%, 65.31%, 57.57% and 53.41% at 15, 30, 45 and 60 DAT, respectively. Zn supplementation (250 mM NaCl + Zn) increased leaf number by 41.02%, 16.48%, 2.61%, and 11.28% at 15, 30, 45, and 60 DAT, respectively, as compared to plants exposed to 250 mM NaCl.

Table 2. *Effects of zinc on leaf number of soybean cv. Shohag at different days after treatment under salinity.*

Treatments	Days after treatment (DAT)			
	15	30	45	60
0 mM NaCl	68.33±19.86 a	81.67±17.50 a	90.33±8.50 a	93.00±10.15 a
50 mM NaCl	59.33±9.87 ab (86.83)	71.67±10.24 ab (87.75)	79.17±2.25 b (87.64)	83.60±5.71 a (89.89)
50 mM NaCl + Zn	67.67±2.04 ab (99.03)	78.33±3.57 ab (95.91)	84.00±3.00 b (92.99)	90.67±2.08 ab (97.49)
100 mM NaCl	52.00±17.98 ab (76.10)	66.33±10.65 abc (81.22)	62.00±7.00 c (68.64)	68.30±8.61 b (73.44)
100 mM NaCl + Zn	58.33±1.53 bc (85.36)	63.33±3.51 bc (77.54)	68.67±4.04 bc (76.02)	82.67±5.50 bc (88.89)
150 mM NaCl	25.00±19.97 b (36.59)	43.33±17.16 abc (53.04)	51.17±3.88 d (56.65)	58.27±9.53 bc (62.66)
150 mM NaCl + Zn	47.67±9.29 cd (69.76)	59.00±6.55 cd (72.24)	62.67±5.68 c (69.38)	68.67±5.09 cd (73.84)
200 mM NaCl	20.00±24.25 b (29.27)	35.00±27.79 bc (42.86)	46.87±1.63d e (51.89)	51.33±2.08 cd (55.19)
200 mM NaCl + Zn	38.00±9.53 de (55.61)	44.00±8.54 de (53.88)	52.33±9.01 cd (57.93)	59.67±7.23 de (64.16)
250 mM NaCl	18.67±9.07 b (27.32)	28.33±13.05 c (34.69)	38.33±3.05 e (42.43)	43.43±3.21 d (46.59)
250 mM NaCl + Zn	26.33±6.66 e (38.53)	33.00±7.55 e (40.41)	39.33±3.52 d (43.54)	48.33±7.02 e (51.97)

* Average value of 9 plants in each treatment.
* Means in a column followed by the same letter do not differ significantly at 5% level and±means standard deviation.
* Values within parenthesis indicate percentage relative to the control.

3.3. Leaf Length

The data on leaf length of soybean cultivar recorded at 15, 30, 45 and 60 DAT are presented in the Table 3. Compared with control, leaf length of plants treated with 200 mM NaCl decreased by 49.91%, 45.89%, 41.47% and 39.45% at 15, 30, 45 and 60 DAT, respectively. Compared to plants subjected to 200 mM NaCl, Zn application (200 mM NaCl + Zn) enhanced leaf length by 24.33%, 6.07%, 2.67% and 4.5% at 15, 30, 45 and 60 DAT, respectively. Compared with control, leaf length of plants treated with 250 mM NaCl decreased by 60.77%, 46.06%, 42.72%, and 40.37% at 15, 30, 45 and 60 DAT, respectively. Compared to plants subjected to 250 mM NaCl, Zn application (250 mM NaCl + Zn) enhanced leaf length by 5.34%, 6.7%, 1.4% and 2.8% at 15, 30, 45 and 60 DAT, respectively.

Table 3. *Effects of zinc on leaf length (cm) of soybean cv Shohag at different days after treatment under salinity.*

Treatments	Days after treatment (DAT)			
	15	30	45	60
0 mM NaCl	10.50±2.65 a	12.16±1.49 a	12.78±1.06 a	13.08±1.29 a
50 mM NaCl	8.74±3.09 a (83.24)	11.10±2.35 ab (91.28)	11.72±1.81 ab (91.70)	12.00±1.74 ab (91.74)
50 mM NaCl + Zn	10.02±2.39 b (95.43)	10.52±2.28 b (86.51)	12.30±0.82 b (96.24)	12.44±0.53 a (95.11)
100 mM NaCl	5.04±0.55 b (48.00)	7.24±1.25 cd (59.54)	8.28±1.42 c (64.79)	8.72±1.50 cd (66.67)
100 mM NaCl + Zn	7.78±1.55 c (74.09)	8.36±1.53 c (68.75)	9.14±1.07 c (71.52)	9.76±0.78 b (74.62)
150 mM NaCl	6.20±1.48 b (57.33)	9.56±1.12 bc (78.62)	10.50±1.54 b (82.16)	10.68±1.69 bc (81.65)
150 mM NaCl + Zn	7.18±1.20 c (68.38)	7.98±0.91 c (65.63)	8.96±1.65 c (70.10)	9.70±1.44 b (74.16)
200 mM NaCl	5.26±1.29 b (50.09)	6.58±1.90 d (54.11)	7.48±1.73 c (58.53)	7.92±1.84 d (60.55)
200 mM NaCl + Zn	6.54±1.32 c (62.29)	6.98±1.35 cd (57.40)	7.68±0.64 d (60.09)	8.28±1.36 c (63.30)
250 mM NaCl	4.12±1.09 b (39.23)	6.56±2.30 d (53.94)	7.32±1.99 c (57.28)	7.80±2.01 d (59.63)
250 mM NaCl + Zn	4.34±1.02 d (41.33)	7.00±1.03 d (57.54)	7.42±0.51 d (58.06)	8.02±0.73 c (61.31)

* Average value of 9 plants in each treatment.
* Means in a column followed by the same letter do not differ significantly at 5% level and±means standard deviation.
* Values within parenthesis indicate percentage relative to the control.

3.4. Leaf Breadth

The data on leaf breadth of soybean cultivar recorded at 15, 30, 45 and 60 DAT are presented in the Table 4. Compared with control, leaf breadth of plants treated with 200 mM NaCl decreased by 44.23%, 41.78%, 40.64% and 36.83% at 15, 30, 45 and 60 DAT, respectively. Compared to plants subjected to 200 mM NaCl, Zn application (200 mM NaCl + Zn) enhanced leaf breadth to 12.76%, 7.06%, 13.01% and 27.57%. Compared with control, leaf breadth of plants treated with 250 mM NaCl decreased by 60.69%, 56.93%, 48.98%, and 46.14% at 15, 30, 45 and 60 DAT, respectively. Compared to plants subjected to 250 mM NaCl, Zn application (250 mM NaCl + Zn) enhanced leaf breadth to 40.63%, 30.15%, 16.73% and 38.60% at 15, 30, 45 and 60 DAT, respectively.

Table 4. *Effects of zinc on leaf breadth (cm) of soybean cv. Shohag at different days after treatment under salinity.*

Treatments	Days after treatment (DAT)			
	15	30	45	60
0 mM NaCl	8.14±1.09 a	9.24±1.22 a	9.84±0.92 a	10.10±0.93 a
50 mM NaCl	7.30±0.84 a (89.68)	7.94±0.80 ab (85.93)	8.40±0.92 b (85.37)	8.98±0.98 ab (88.91)
50 mM NaCl + Zn	6.98±0.89 ab (85.75)	7.86±0.92 ab (85.06)	8.26±0.87 ab (83.94)	8.86±0.99 ab (87.72)
100 mM NaCl	5.52±1.62 b (67.81)	6.52±1.68 bc (70.56)	7.24±1.75 b (73.58)	7.7±1.74 ab (76.24)
100 mM NaCl + Zn	6.30±1.67 abc (77.39)	7.28±1.61 ab (78.79)	7.66±1.81 abc (77.85)	6.98±0.91 ab (69.11)
150 mM NaCl	4.26±0.85 bc (52.33)	5.08±0.79 cd (54.98)	5.8±0.94 cd (58.94)	6.44±0.99 bc (63.76)
150 mM NaCl + Zn	5.58±1.50 bc (68.55)	6.46±1.49 bc (69.91)	7.10±1.59 bc (72.15)	6.14±1.29 bc (60.79)
200 mM NaCl	4.54±1.36 bc (55.77)	5.38±1.31 cd (58.22)	5.84±1.15 cd (59.36)	6.38±1.20 bc (63.17)
200 mM NaCl + Zn	5.12±1.60 bc (62.89)	5.76±1.37 cd (62.33)	6.60±1.11 bc (67.07)	8.14±1.74 bc (80.59)
250 mM NaCl	3.20±0.84 c (39.31)	3.98±0.62 d (43.07)	5.02±0.55 d (51.02)	5.44±0.48 c (53.86)
250 mM NaCl + Zn	4.50±1.31 c (55.28)	5.18±1.27 d (56.06)	5.86±1.14 c (59.55)	7.54±1.54 c (74.65)

* Average value of 9 plants in each treatment.
* Means in a column followed by the same letter do not differ significantly at 5% level and±means standard deviation.
* Values within parenthesis indicate percentage relative to the control.

3.5. Leaf Area

The data on leaf area of soybean cultivar recorded at 15, 30, 45 and 60 DAT are presented in the Table 5. Compared with control leaf area of plant treated with 200 mM NaCl decreased by 72.62%, 68.38%, 65.52% and 61.94% at 15, 30, 45 and 60 DAT, respectively. Compared to plants subjected to 200 mM NaCl, Zn application (200 mM NaCl + Zn) enhanced leaf area by 43.81%, 14.22%, 17.32% and 15.05% at 15, 30, 45 and 60 DAT, respectively. Compared with control, leaf area of plant treated with 250 mM NaCl decreased by 84.31%, 76.67%, 70.61%, and 67.96% at 15, 30, 45 and 60 DAT, respectively. Compared to plants subjected to 250 mM NaCl, Zn application (250 mM NaCl + Zn) enhanced leaf area by 41.85%, 40.09%, 18.47% and 17.67% at 15, 30, 45 and 60 DAT, respectively.

Table 5. *Effects of zinc on leaf area (cm^2/plant) of soybean cv. Shohag at different days after treatment under salinity.*

Treatments	Days after treatment (DAT)			
	15	30	45	60
0 mM NaCl	85.29±23.57 a	111.85±16.39 a	125.07±6.07 a	131.41±9.95 a
50 mM NaCl	65.00±26.11 b (76.21)	89.58±25.23 b (80.08)	98.16±17.26 b (78.48)	108.99±14.68 b (82.93)
50 mM NaCl + Zn	69.56±17.18 b (81.56)	82.61±20.81 b (73.86)	101.79±14.71 a (81.39)	110.02±10.93 b (83.73)
100 mM NaCl	27.24±5.84 c (31.94)	47.87±17.72 c (42.79)	60.29±20.31 c (48.20)	67.92±23.64 c (51.69)
100 mM NaCl + Zn	46.03±16.47 c (53.97)	58.61±16.23 c (52.40)	70.53±19.69 b (56.39)	79.78±19.29 c (60.71)
150 mM NaCl	26.36±7.59 c (30.91)	48.49±9.40 c (43.35)	60.60±12.09 c (48.45)	68.18±12.27 c (51.89)
150 mM NaCl + Zn	45.03±20.47 c (52.79)	55.57±22.44 c (49.68)	65.31±25.74 bc (52.22)	73.38±20.49 c (55.84)
200 mM NaCl	23.35±7.85 c (27.38)	35.37±14.18 c (31.62)	43.12±11.31 cd (34.48)	50.01±13.72 cd (38.06)
200 mM NaCl + Zn	33.58±13.39 cd (39.37)	40.40±13.38 cd (35.47)	50.59±8.92 bc (40.44)	57.54±9.75 cd (43.78)
250 mM NaCl	13.38±5.54 c (15.69)	25.09±9.86c (23.33)	36.77±10.84 d (29.39)	42.10±9.39 d (32.04)
250 mM NaCl + Zn	18.98±4.71 d (22.25)	35.15±5.55 d (31.42)	43.56±9.62 c (34.82)	49.54±12.91 d (37.69)

* Average value of 9 plants in each treatment.
* Means in a column followed by the same letter do not differ significantly at 5% level and±means standard deviation.
* Values within parenthesis indicate percentage relative to the control.

4. Discussion

It was observed that the height of soybean plants decreased as the salinity level increased. Plant height reductions due to salinity has been reported in rice, [21], soybean [20], lemon balm [22, 23]. Mazher *et al.* [24] stated that the detrimental effect of salt on photosynthesis, changes in enzyme activity and a decrease in carbohydrate and growth hormone levels could all contribute to the salt-induced reduction in plant height. Results showed that zinc application improved shoot and root length under all salinity treatments. Similar results have been reported by Ahmad *et al*. [16] and Weisany *et al*. 25]. Zinc enhances plant growth by stimulating cell division and proliferation by increasing natural auxin synthesis [26]. Moreover, proper concentration of zinc plays a critical role in membrane permeability, phospholipid accumulation and reactive oxygen species (ROS) detoxification under salinity [17, 27].

With increasing salinity, the production of leaf numbers was negatively impacted. Since the leaf is the primary photosynthetic organ, the leaf number is critical for plant development. Similar results of reduction in leaf number under saline conditions have been reported in rice [21], in purslane [28] and barley [29]. Under salt stress, the formation of leaf primordial is inhibited, which may be the cause of low leaf number [30]. Additionally, the accumulation of sodium chloride in the cell walls and cytoplasm of older leaves may be causing the decline in leaf numbers. In contrast, zinc application increased leaf number in all salinity treatments. Similar result has also been found by Sarhan *et al*. [31]. Jiang *et al*. [32] demonstrated that Zn could aid nutrient translocations from old to new cells.

In this study, the leaf length and leaf breadth were adversely affected by salinity. The reduction in leaf length and leaf breadth were quite incremental at all salinity levels. The results are in confirmation with the findings of Ashrafuzzaman *et al.* [33]. However, when zinc was applied, leaf length and leaf breadth increased under salinity stress.

It is obvious that production of photosynthetic area is disturbed by abiotic stress [34]. As a result, the overall growth and development of a plant is severely disrupted. The decrease in green leaf area could be attributed to increased leaf senescence as well as smaller leaves formed under saline conditions. Several authors have been reported that leaf area per plant decreased markedly by salinity in rice [35, 36] in wheat [37, 38], in maize [33], in bean [39], black gram and mung bean [40]. Whereas, zinc application enhanced leaf area under all salinity treatments. Many scientists stated that applying Zn to crop plants in a saline environment improved leaf area [31, 41, 42]. It has been concluded that Zn nutrition may reduce negative salinity effects by promoting plant Zn/K intake while restricting plant Na storage, resulting in increased leaf cell membrane stability, chlorophyll synthesis, and leaf water retention [43, 44].

5. Conclusions

This study concluded that salt stress negatively affects plant growth in terms of plant height, leaf number, leaf length, leaf breadth and leaf area. It can also be concluded that zinc and salinity interaction provides comparatively greater plant growth in soybean plants than salt stress alone. Overall, the findings suggest that zinc supplementation may reduce the harmful consequences of NaCl stress. Therefore, future experiments will be conducted to determine the mechanisms underlying the enhanced safety of soybean against the harmful effects of salt due to zinc application.

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