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Effect of cadaverine on *Brassica juncea* (L.) under multiple stress

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The cadaverine (Cad), an organic diamine was examined for its response on growth in salinity and metal stressed *B. juncea* cv RH-30 vis-à-vis compared the response of ammonium nitrate. The Cad (1 mM) application ameliorated the effect caused by salinity and metal stress on seed germination and plant growth. The plant growth recovery (dry biomass accumulation) was dependent on stress and diamine type. The higher growth recovery potential of Cad under both stresses was due to elevation in photosynthetic pigments, nitrate reductase activity and organic nitrogen as well as soluble protein, It is inferred that growth in stressed seedlings was mediated by Cad through lowering endogenous Cd/Pb and Na^+/K^+ level in leaf and shoot tissues.

Keywords: *Brassica juncea*, Cadaverine, Metal, Salinity, Stress

The damaging effects of some of the stresses could be mitigated by exogenous application of polyamines (PAs). The anti-stress role of PAs[1,2] and putrescine (Put)[3-5] have been suggested in many plants. However, differential accumulation of PAs considered as marker under stresses, depends on developmental conditions[6] and age of the plant[7]. The cadaverine (Cad), structurally different diamine and having independent biosynthetic pathway in relation to Put, also grouped in the family of polyamines regarded as a growth regulator[8,9] and proposed same mode of action[10]. Like Put the Cad also tends to accumulate in higher plants under osmotic/salt stresses[11] but without explicit explanation of its role in specific stress alleviation. In view of some conflicting observations it is pertinent to peruse that Cad inspite of structural difference with that of Put or ammonium nitrate might pose some different responses in plants under different conditions. Salinity has no relationship with Cad level in *Oryza sativa*[12]. While, there is either little change in maize[13] or decrease in wheat, or no accumulation in barley and *Vicia faba*[14,15]. Aziz *et al.*[16,17] have demonstrated Cad accumulation in osmotically stressed leaf disc of rape and tomato under salinity.

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Carrizo *et al.*[18] have demonstrated age dependent Cad titer, which declined progressively in *Brugmansia candida.* The PAs in general have been assigned as an antisenescence molecules[11,16,19] along with Put[3,5]. However, Cad is implicated in differential alkaloid synthesis in plants[18]. Few biological roles of Cad have been demonstrated during oxidative stress[19-21]. Thus, the Cad exogenous application under stress likely to exert some positive response in stressed plants, which is not elucidated well. Therefore, the present study has been undertaken to provide some insight to understand its Cad's regulatory mechanism in plants growth and its potential to mitigate the adverse responses induced under multiple stresses, generally realized by plants in field condition vis-a–vis to compare its response with NH_4NO_3.

Materials and Methods

Seeds of mustard (*Brassica juncea* L. cv RH-30) were surface sterilized with bleaching powder ($CaOCl_2$) for five minutes and sown in Whatman's filter paper lined petri-plates. The seedlings were grown in controlled condition (light 75 Wm^2, 25±2 ºC, 65% RH) and watered with half-strength Hoagland nutrient solution containing NaCl (100 m*M*), Cd or Pb (1 m*M*) and blended with Cad (1 m*M*) and NH_4NO_3 (5 m*M*) as per experimental plan. The *p*H 6.4 of the nutrient solution (containing salts and cadaverine) was kept constant for all treatments (T0: Control, T1: NaCl, T2: NH_4NO_3, T3: NaCl+NH_4NO_3, T4: Cd, T5: Pb, T6:

Cd+NaCl, T7: Pb+NaCl, T8: Cd+NaCl+ NH_4NO_3, T9: Pb+NaCl+NH_4NO_3) with and without Cad. The seed germination was counted after 24 and 48 h of sowing. Random sampling of morphologically and physiologically similar seedlings was done on 7th day for various measurements. Biomass was estimated after drying the seedlings for 72 h at 68 ºC. Estimation of chlorophyll contents[22], carotenoid[23], nitrate reductase activity (*in vivo*)[24] and organic nitrogen[25] was done. The Na^+ and K^+ were estimated by using Digital Flame Photometer and Pb and Cd by atomic absorption spectrophotometer (Shimadzu 660-13). The data are mean of three replicates along with ±SD. For the significance of the treatments data analyses was done using Student's '*t*' test.

Results

Germination and growth under multiple stress with and without Cad—Either salinity or Pb or Cd alone or in combination reduced the seed germination examined after 24 h (Fig. 1). The germination patterns of seeds under each type of stress after 48 h were almost remained same. But salinity and metal stress in combination decreased the germination further. The Cad application showed protective effect on seeds germinability under all stress except Pb plus salinity (T7). The pattern of root/shoot elongation in the seedling was similar under any of the stress, salinity or metal alone or in combination with or without Cad treatment (Fig. 2). However, the Cd alone and in combination with salinity caused severe reduction in root/shoot elongation as compared to that of Pb and Pb plus salinity. The Cad reversion of shoot/root length reduction was not prominent under Cd stress in comparison to Pb plus salinity.

The Cad in combination with NH_4NO_3 did not exert any synergistic effect on elongation of the seedling. The seedling's growth was protected by Cad under multiple stress (Fig. 3). Interestingly, the root growth was greatly protected by Cad under multiple stresses than the shoot growth. There was increase in accumulation of biomass in root with NH_4NO_3 in the presence of Cad (unpublished data). The relative growth of seedlings examined from 6th to 9th day was found in decreasing order in salt stressed plant (unpublished data). Supplementation of NH_4NO_3 did

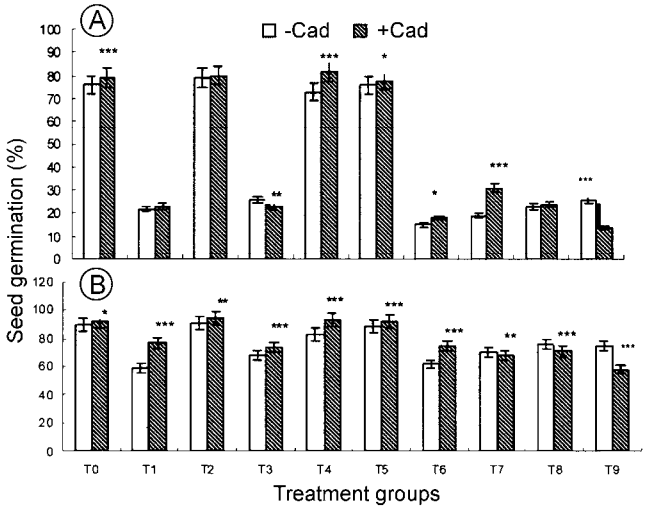

Fig. 1—Mustard seed germination under salinity, metal and twin stress with or without Cad after 24 h (A) and 48 h (B) T0 = Control, T1 = NaCl (100 mM), T2 = NH_4NO_3 (5 mM), T3 = NaCl+NH_4NO_3, T4 = Cd (1 mM), T5 = Pb (1 mM), T6 = Cd+NaCl, T7 = Pb+NaCl, T8-Cd+NaCl+NH_4NO_3, T9 = Pb+NaCl+NH_4NO_3.Values are mean± SD from 3 replicates. *P* values: *<0.05%, **<0.01, ***<0.001]

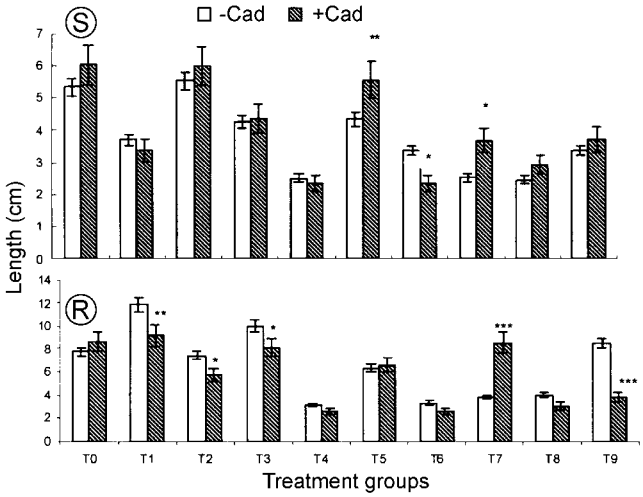

Fig. 2—Root (R) and shoot (S) length under stress on 7th day old seedlings with or without Cad (other details are same as in Fig. 1).

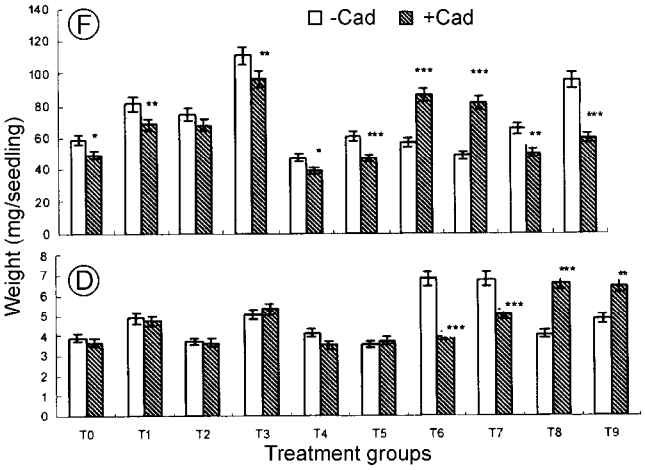

Fig. 3—Fresh (F) and dry (D) weight of seedling on 7th day under salt (upper panel) and metal (lower panel) stress with or without Cad (other details are same as in Fig. 1).

not alter in growth rate, in stressed seedlings which changed little under multiple stresses, while Cad promoted the growth rate.

Photosynthetic pigments under multiple stress—Total chlorophyll content in leaf tissues increased with Cad treatment over control. Pb plus NaCl exposed plants treated with NH_4NO_3 showed higher content of chlorophyll, which improved with Cad supplementation. The Cad treatment of the seedlings elevated the carotenoid content also in the seedlings under all stress conditions except the Pb stressed seedlings (T5) and remained consistent under multiple stresses also (Fig. 4).

Nitrate reductase activity (NRA) *in vivo under multiple stress*—Interestingly root has higher level of NRA than leaf of *B. juncea* (Fig. 5), which declined under all kind of stress (T1, T4, T5 alone and in combination T6, T7). However, NRA in leaf and root tissues increased considerably by Cad and with NH_4NO_3 under stress also (Fig. 5). Though, Cad effect on NRA in either tissue under multiple stress was not prominent. The presence of NH_4NO_3, Cad effect on NRA in leaf tissue was not observed, while NH_4NO_3 improved the activity significantly. The induction of NR activity in the root by Cad in the Pb environment was almost doubled.

Total organic nitrogen in tissues of seedlings—The total organic nitrogen in the tissues of the seedling exposed to stresses in the presence of Cad showed comparatively little change in the leaf as compared to root/shoot except when supplemented along with NH_4NO_3 (Fig. 6). But, the total organic nitrogen accumulation with Cad in root of seedlings under multiple stresses (T8, T9) increased dramatically. The decline in total organic nitrogen in shoot and root tissues of the seedlings under twin stresses was reverted with Cad treatment. The Cad response on total organic nitrogen in leaf was consistent in each treatment.

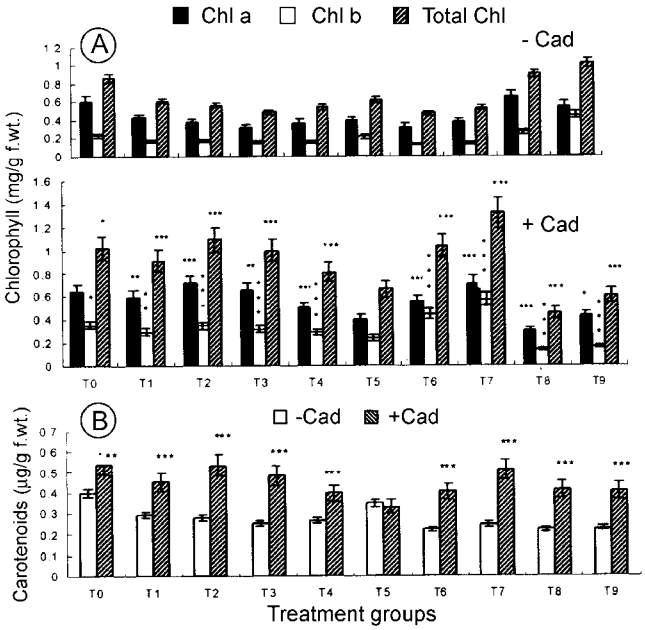

Fig. 4—Chlorophyll (A) and carotenoid (B) content in leaf tissues of 7th day old seedlings under stress with or without Cad (other details are same as in Fig. 1).

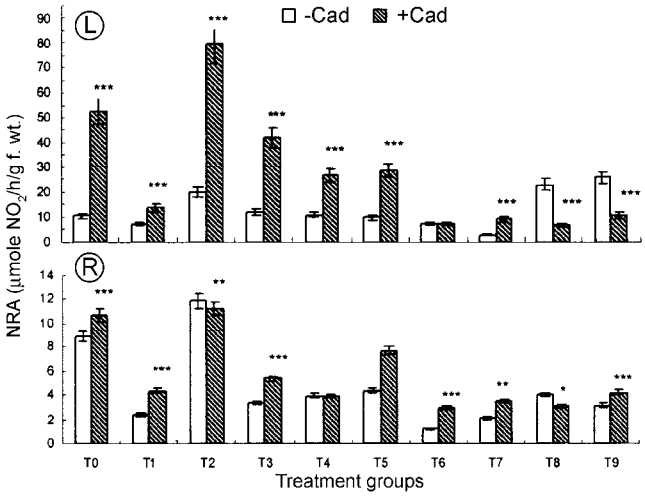

Fig. 5—Nitrate reductase activity (*in vivo*) in the leaf (L) and root (R) tissues of 7th day old seedlings under stress with or without Cad (other details are same as in Fig. 1).

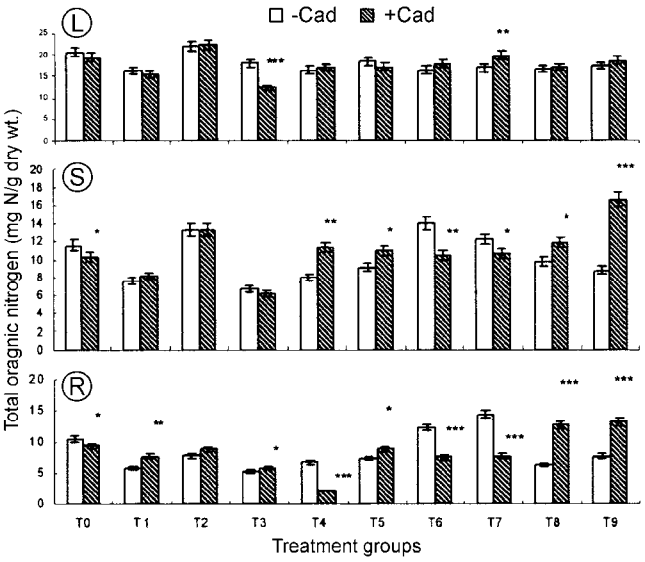

Fig. 6—Total organic nitrogen content in the leaf (L), stem (S) and root (R) tissues of 7th day old seedlings grown under stress with or without Cad (other details are same as in Fig. 1).

Na^+/ K^+ in tissues with and without Cad—Salinity stress elevated the Na^+ level in leaf tissues about 2 fold over control. Surprisingly, the trend of rising of Na^+ level did not change with NH_4NO_3 treatment. The Cad supplementation also did not influence endogenous Na^+ level in multiple conditions. The Cad caused little decline only under salinity stress.

The tissues K^+ level was also reduced under salinity or metal stress over control. However, the NH_4NO_3 treated plants showed a very high level of K^+ in leaf over control and that remained almost higher even when such plants were stressed with salinity. Unlike salt exposed plants, the salinity in presence of Cd stressed plants also exhibited elevation in K^+ level over control, but the Pb combination of salinity depressed the endogenous K^+ level in leaf. Further, the multiple stressed plants with NH_4NO_3 did also show elevation in the K^+ level in leaf tissues (Fig. 7). Cad supplementation however, reduced the K^+ level in respective plants under all stress conditions except Pb exposed plants where K^+ level was raised over only Pb exposed plants.

The stem tissues were also analyzed for Na^+ and K^+ level in those plants exposed to different magnitude of stresses (Fig. 7). The stem Na^+ contents were generally low compared with that of leaf, also changed depending upon kind of stress exposure to the plants. The Cad treatment of those stressed plants increased the Na^+ content in each case which was unlike to leaf tissues.

On the other hand, K^+ content was quite prominent in stem tissues compared with that of leaf (Fig. 7). The various stress conditions reduced the K^+ level in the stem tissues depending upon magnitude of stress applied. Further, Cad supplementation reduced the K^+ content increase in control, salinity and salinity plus NH_4NO_3 treated plants. Cd or Pb or their combination with salt exposed plants showed reduction in Na^+ content, which was also checked by Cad (Fig. 7). Root tissues had 2-3 folds more Na^+ level than leaf tissues (Fig. 7). But the level declined under the multiple stress conditions. Interestingly, the K^+ level was lowered in each kind of treatment irrespective of Cad or NH_4NO_3 presence.

Cd/Pb level under multiple stress with and without Cad—The endogenous load of Cd/Pb in root tissue was higher than stem and leaf of the seedling exposed to metal stress (Fig. 8). The magnitude of tissues metal accumulation did not change much with salinity in case of Pb.

The Cad application reduced Cd content under each type of stress conditions. However, the Pb load in leaf tissue was lower than that of Cd. The Cad response on Pb/Cd accumulations in each tissue remained same.

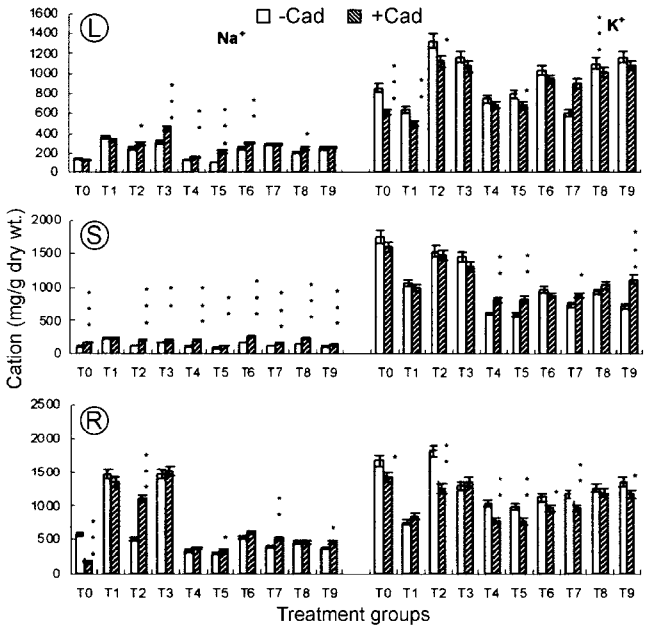

Fig. 7—Na^+ and K^+ content in the leaf (L), stem (S)and root (R)tissues of 7th day old seedlings under stress with or without Cad (other details are same as in Fig. 1).

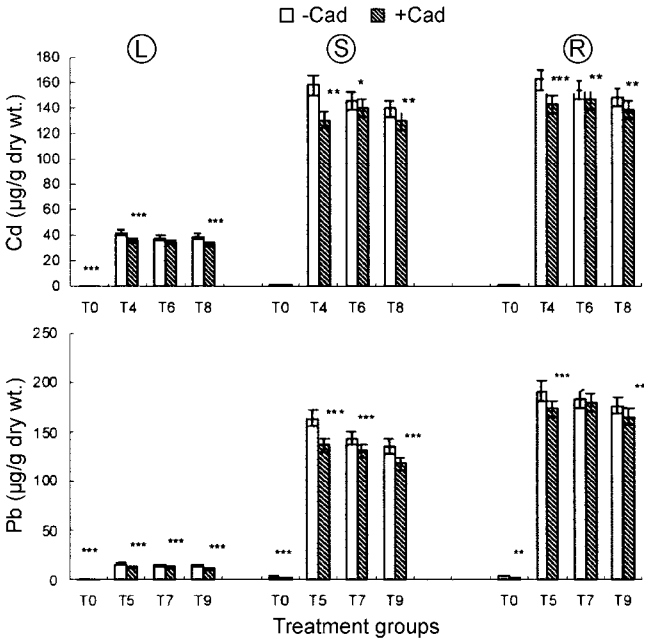

Fig. 8—Endogenous Cd and Pb content in the leaf (L), stem (S) and root (R) tissues of 7th day old seedlings under salinity and metal stress with or without Cad (other details are same as in Fig.1).

Discussion

The stress condition singly or in combination inhibits physiological process depending on stress type. The Cad controlled the inhibition in germination and growth caused by stresses (Fig. 1). Non responsiveness of Cad in the presence of Pb plus salinity (T7) may be because of strong inhibition of hydrolytic enzymes involved in germination or excessive accumulation of Pb and ions in the embryonic tissues[26]. Kakkar and Rai[27] have mentioned that NH_4NO_3 or Cad may improve germination up to some extent only. Cad supplementation to the stressed plants showed positive effect on shoots and root length depending on severity of stress (Fig. 2). Seedlings root tend to increase the surface area to cope with water scarcity under salinity. However, NH_4NO_3 and Cad alone or together showed varied response. The decreased growth rate under salinity was not changed with supplementation of NH_4NO_3 while vice-versa with Cad.

The overall growth inhibition by stress linked with chlorophyll and carotenoids decline in leaves[28, 29] was checked significantly with Cad or NH_4NO_3 (Fig. 4). This Cad potential of growth up regulation seems to be like that of putrescine[3,4]. The Cad modulation of stressed plant growth might be through stimulation of NRA (Fig. 5). The putrescine protects the enzyme activity from both Pb and Cd toxicity even blended with salinity[30] and by other polyamines in maize[31]. The NRA is positively correlated with organic nitrogen in plants[32]. The higher level of NRA with Cad in root tissues noticeable in this plant (Fig. 5) apparently indicates the potential contribution of root in plant nitrogen status. The results also showed that NRA was very sensitive with either Cad or NH_4NO_3, and the elevated activity could be due to *de novo* synthesis of protein, which was pronounced in leaf (Fig. 5). The Cad induced increase in total organic nitrogen content which otherwise declined in stress condition (Fig. 6) might be linked with NRA. The organic nitrogen status plays a key role in crop development which is considered to be crucial for CO_2 fixation and dry matter production[33] seems to be modulated by Cad, as mentioned in case of other plant growth regulators[34]. The growth modulation in stress plant also depends on ion homeostasis in general and under salinity in specific. The Na^+/K^+ major player of the ionic homeostasis, changed in *B. juncea* root more drastically than stem and leaf tissue (Fig 7). It was surprising that Cd or Pb did not modify the Na^+ level; even twin stress was applied to the seedlings. Probably the Na^+ transporters disturbed with alteration of membrane permeability was not further altered with metal and neither was it reverted by either of the nitrogen source supplementation. Almost similar response of K^+ under stress and with Cad also indicated this behaviour. Such response was also noticed with putrescine[3,4] suggesting that both diamine might have similar mode of action on ion homeostasis in stressed plants. The Na^+/K^+ content change pattern in the whole seedling under stress with Cad did not change significantly. This showed that Cad had no specific effect on tissue partitioning of Na^+ and K^+. The Na^+ competition for K^+ uptake system might inhibit K^+-dependent processes, therefore low Na^+ and high K^+ cytoplasmic content is suggested for plant growth[35], which is being maintained by Cad results into growth (Fig. 7). The Cad response on plant growth under Cd/Pb along with salinity stress corroborates with the response with GA_3 in wheat under salt stress[36]. The Cad and NH_4NO_3 checked progressive decrease in growth (Fig. 3) and certain metabolites (Fig. 4) in plants under higher Pb or Cd load in shoot and leaf of the seedlings (Fig. 8) like in other cases[37] was indicator of overall physiochemical modulation. The higher translocation of Cd than Pb in shoot to leaf under stress was also lowered by Cad, indicating elevated potential of RH-30 as "shoot excluder" of toxic metals. However, the differential accumulation pattern of Cd and Pb was similar to that of rice[35]. The salinity enhanced metal accumulation may be due to change in the bio-membranes[38] which otherwise might be protected by NH_4NO_3 or Cad. Verma and Mishra,[39] have suggested that putrescine stabilize the membrane of the plants stressed with salinity. It appears that the Cad strongly maintains the stressed plant growth through maintaining the metabolites and ionic homeostasis.

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