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EVALUATION OF SALT TOLERANCE OF MUNGBEAN CULTIVARS AT THE SEEDLING STAGE USING POTENTIAL GROWTH INDICES

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Abstract

Soil salinity is a significant abiotic factor that lowers crop productivity. In the sub-tropical countries of Asia, mungbean is grown as a marginal crop in rice-based agricultural ecosystems, where salts get deposit in the upper layer. Therefore, robust criteria are essential to screen high yielding salt-tolerant cultivars of mungbean. The current study is an effort to assess the salt tolerance potential of twelve mungbean genotypes under laboratory environment at control (0), 50, 75, and 100 mM NaCl salinity level using stress tolerance indices. The findings showed that in all cultivars, with the increase in level of stress there is a significant reduction in the germination (GSI), fresh (FWSI) and dry biomass (DWSI), length of root (SLSI) and shoot stress tolerance indices (RLSI). NM-2021 showed 98% GSI followed by AbbasMung (89%), however, NM-54 showed the lowest value for GSI (62%). NM-98 exhibited 74% FWSI followed by Abbas Mung 73%. Abbas Mung was at the top with 82% DWSI followed by NM 19-19 (81%). NM 19-19 exhibited the highest value for SLSI (69%) and RLSI (81%). There is a correlation between the fresh and dry biomass tolerance indices of seedlings. The data was categorized into three clusters using the multivariate cluster analysis approach in order to assess the similarities between mungbean cultivars. Cluster-I exhibited the salt-tolerant cultivars NM-2021, NM-98, NM-92, Abbas Mung and NM-19-19, Cluster-II classified medium-tolerant cultivars NM-121-2, NM-2021, NM-2006 and NM-2016, and Cluster-III showed salt-sensitive cultivars NM-51, NM-2011 and NM-54. The growth indicators could be utilized as selection criteria to assess salt tolerance in mungbean cultivars.

Key words: NaCl, Tolerance indexes, Dendrogram, *Vigna radiata*.

Introduction

The phenomenon of soil salinity is the most significant stress factor impeding plant development. It causes delay in seed germination and ultimately reduces agricultural yield. In dried as well as semi-dried land of the world, salt stress affects plant diversity and agricultural productivity, a major risk to food safety (Farooq *et al*., 2015). In Pakistan 6.3 x 10^6 hectares of the land is salt affected (Qureshi, 2016). According to the estimates by Pitman & Läuchli (2002), over twenty percent of the world's irrigated agricultural area is salt affected. Therefore, in order to achieve high yield under saline soils, it is essential to use conventional as well as contemporary methods to create salt tolerant cultivars (Ullah *et al*., 2022).

Elevated salt concentration in soil makes it challenging for roots to uptake water, causing physiological drought and toxicity of ions due to uptake of specific ions in plants. More Na^+ uptake disturbs the metabolism of plants due to the generation of reactive oxygen species, oxidative damage of cellular processes, proteins and nucleic acids leading to cell death (Ali *et al*., 2022). The saline lands are expanding greatly owing to poor drainage and irrigation. The foremost injurious effect of NaCl stress is ionic imbalance due to Na^+ and Cl^- buildup and inhibition of K^+ uptake causing low productivity and ultimately cell death of crops (Zafar *et al*., 2015; Mohsan *et al*., 2022).

To acquire economic output from saline soils, screening of germplasm is a fundamental prerequisite before choosing salt-tolerant cultivars. A selection criterion must be employed as an assessment method to assess the ranges at the initial sowing stage instead of evaluating them at the yielding stage (Hanumantha Rao *et al*., 2016: Negrão *et al*., 2017). Physiological indicators can be used to analyze genetic differences for stress tolerance in crop plants during the stages of sprouting and initial development. *Triticum aestivum* L. has been successfully screened for its ability to tolerate salt, and this tolerance may be determined at the seedling stage for both the vegetative and reproductive phases (Miransari & Smith, 2019).

Vigna radiata L. commonly known as mungbean is a pulse crop that is grown on marginal lands, as fertile lands are largely taken up by crops with high income returns (Sheoran *et al*., 2022). It contains high amount of vitamins, proteins and minerals. Salt stress negatively affects mungbean growth, performance and quality due to growth arrest and metabolic disorders ultimately resulting in cell death and significant loss in yield and revenue (Sehrawat *et al*., 2019). Therefore, screening of salt-tolerant cultivars is very crucial for the increase of mungbean yield in saline environment.

The creation of high yielding salt-tolerant mungbean cultivars for the productive agriculture of saline regions may benefit from the examination of mungbean germplasm. The dissimilarity among the cultivars for the growth indices at germination and early seedling has been evaluated in many crop plants. This aids in classifying the tolerant cultivars, which can be studied and economically exploited for cultivation on salt-affected lands (Zafar *et al*., 2015). Our study is an attempt to compare the usefulness of several stress indices for identification of cultivars with better performance at different levels of salt stress. The objective of the present study was to evaluate 12 mungbean cultivars under NaCl stress and to assess the variability in their genotypic response to salinity at an early seedling stage.

Material and Method

Experiment details: The experiment was performed under laboratory conditions in a growth chamber in Soil Biology Division, NIAB, Faisalabad. Seeds of twelve cultivars of mungbean NM-2021, NM-98, NM-92, Abbas Mung and NM-19-19, NM-121-2, NM-2021, NM-2006, NM-2016, NM-51, NM-2011 and NM-54 were surface sterilized with 10% sodium hypochlorite (NaClO) and washed three times with distilled water. They were allowed to germinate under four levels 0, 50, 75, and 100 mM of sodium chloride in a growth chamber with 80 µM S^{-1} m^{-2} light intensity and temperature set at 27°C ± 2°C. The germination of seeds was recorded on day-to-day basis and after two weeks, all the seedlings were taken out. The length and biomass of the mung bean plant was recorded. The plants were oven dried at 72°C for 45 hours for the determination of dry weight (Zafar *et al*., 2015). Physiological indices were calculated and were used as a screening technique in the current experiment to determine the potential for stress tolerance of twelve different mungbean cultivars. Once the radical's length extended by 5 mm, germination was observed.

Physiological indices: Daily counts of the seeds that germinated were made, and the promptness index (PI) was determined. It was used to compute the germination stress index on the initial, 2nd, 3rd, and 4th days, accordingly.

Promptnex index = Number of seeds day1 (1.00) + Number of seeds day 2 (0.75) + Number of seeds day 3 (0.50) + Number of seeds day 4 (0.25)

Stress indices were calculated using the following formula:

$$\text{Germination stress index} = \frac{\text{PI of seeds under stress}}{\text{PI of control seeds}} \times 100$$

$$\text{Shoot length (SL) stress index} = \frac{\text{SL of plants under stress}}{\text{SL of control plants}} \times 100$$

$$\text{Root length (RL) stress index} = \frac{\text{RL of plants under stress}}{\text{RL of control plants}} \times 100$$

$$\text{Fresh weight (FW) stress index} = \frac{\text{FW of plants under stress}}{\text{FW of control plants}} \times 100$$

$$\text{Dry weight (DW) stress index} = \frac{\text{DW of plants under stress}}{\text{DW of control plants}} \times 100$$

Statistical analysis

The acquired data underwent statistical analysis (ANOVA), and the Least significant difference test and a 5% probability level was used to compare the means (Steel *et al*., 1997). The Minitab-6 was used to perform cluster analysis.

Results

All mungbean cultivars exhibited reduced seed germination under salinity stress. Under 50, 75 and 100 mM NaCl levels, the GSI was (99%), (78%), and (70%) (Table 1) respectively. Cultivar NM-54 had the lowest GSI (73%, 60, 53%) and cultivar NM-2021 had the highest (100%, 100, and 93%) at 50, 75, and 100 mM NaCl, accordingly. According to the cultivar means, NM-54 was in last place, followed by NM-2011 and NM-2006 and NM-2051.

Table 1. Germination stress tolerance index of mungbean cultivars.

Cultivars	NaCl stress in Mm				Ranking
	50	**75**	**100**	**Mean** (%)	
NM-2021	100	100	93.33	97.778a	1
NM 121-25	100	80	73.33	84.444b-d	4
NM-92	100	86.66	73.33	86.667bc	3
Abbas Mung	100	86.66	80	88.889b	2
NM 19-19	100	86.66	80	88.889b	2
NM-51	86.66	66.66	60	71.111ef	7
NM-54	73.33	60	53.33	62.222g	9
NM-98	100	86.66	80	88.889b	2
NM-2011	86.66	66.66	46.66	66.667fg	8
Nm-13-1	80	80	73.33	77.778de	6
NM-2006	73.33	73.33	66.66	71.111ef	7
NM-2016	100	73.33	66.66	80.000cd	5
Mean	99.99a	78.80b	70.55c		

Note: Means with same letter did not vary significantly (p>0.05) in row and column

All mungbean cultivars experienced a considerable reduction in shoot and root length (SLSI and RLSI) (Tables 4, 5) under salinity stress. An increase in salinity caused a significant decrease in SLSI. Overall means showed 71%, 51%, and 44% values of SLSI under 50 mM, 75 mM and 100 mM NaCl stress respectively. However, regarding RLSI the overall means showed 71%, 56, and 40% values of RLSI for the mungbean cultivars (Tables 4,5). The cultivars NM-54 (38.5%) and NM-2011 (40.9%) displayed the lowest SLSI above 50 mM sodium chloride, while variant NM-19-19 displayed the greatest SLSI (68%) and was closely followed by Abbas Mung (66%). At 100 mM stress of NaCl, cultivar NM-54 had the lowest SLSI (24.2%), while cultivar NM-19-19 was able to maintain the maximum SLSI (60.3%). According to the total ranking and cultivar means, NM-19-19 was at the highest rank, whereas NM-54 and NM-2011 were at the lowest. Similarly, NM-54 showed the lowest RLSI value (66.05%) at a 50 mM NaCl level, where NM-19-19 displayed the highest value (90%). Cultivar-NM-54 had the lowest RLSI (35.18%) while NM-1919 had the greatest RLSI (81%) at 100 mM NaCl. According to variance means, NM-19-19 and NM-92 were placed first and second, respectively, with NM-54 at position 11. FWSI was significantly decreased at 50, 75, and 100 mM NaCl stress (84%, 71.5, and 54%, respectively). At 75 mM NaCl stress, NM-19-19 displayed the highest FWSI (76%), although NM-51 had the lowest FWSI (67%). At 100 mM NaCl, NM 19-19 showed the uppermost FWSI (73%) while NM-2016 showed the lowest FWSI value (57%). The NM-92 and RAMZAN got the highest possible scores for the FWSI and were ranked first and second, respectively, whereas the NM-2016 was ranked ninth (Table 2).

The DWSI of all cultivars meaningfully decreased at 50, 75, and 100 mM NaCl stress, corresponding to 79%, 59%, and 52%, respectively. At a NaCl concentration of 50 mM, Abbas Mung had the highest DWSI (100%), and NM-98 had the lowest (64%) value. Under 100 mM NaCl salinity, NM-121-25 had the uppermost estimated DWSI (72%) although NM-54 had the lowest (24%). According to the averages, Abbas Mung did the best and placed first, whereas NM-54 had the last DWSI scores and was ranked in 9th place (Table 3).

The mungbean cultivars were divided into three clusters in the current cluster analysis dendrogram according to their salt tolerance (Fig. 1). Cluster 1 contained salt resistant cultivars NM-2021, NM-98, NM-92, Abbas Mung and NM-19-19, Cluster 2 contained medium tolerant cultivars NM-121-2, NM-2021, NM-2006 and NM-2016, and Cluster 3 contained salt sensitive cultivars Cluster 3 NM-51, NM-2011 and NM-54.

Table 2. Fresh weight stress tolerance index of mungbean cultivars.

Cultivars	NaCl stress in Mm				Ranking
	50	75	100	Mean	
NM-2021	85.3185	72.5351	53.7656	70.539a-d	4
NM 121-25	91.1312	77.4635	54.0718	74.222ab	2
NM-92	86.5297	73.3083	55.9068	71.914a-c	3
Abbas Mung	87.8209	75.2915	54.5855	72.565ab	2
NM 19-19	85.4366	76.4595	59.1426	73.6795ab	2
NM-51	83.8933	67.8021	44.7517	65.482c-e	6
NM-54	87.8623	70.7276	44.6296	67.739b-e	5
NM-98	88.0406	75.9147	59.5172	74.491a	1
NM-2011	80.685	68.0887	44.0515	64.275de	7
Nm-13-1	88.1793	73.5377	52.6956	71.471a-c	3
NM-2006	73.4996	65.8184	44.8873	61.4018ef	8
NM-2016	74.2003	62.0064	35.8965	57.368f	9
Mean	84.37a	71.58b	54.03c		

Note: Means with same letter did not vary significantly (p>0.05) in row and column

Discussion

The research findings showed that under saline surroundings, all cultivars showed a considerable decrease in development indices such as GSI, SLSI, RLSI, FWSI, DWSI (Tables 1–5). All mungbean cultivars exhibited a significant decline in the seed germination stress indices at increasing salinity levels (Table 1). These results correspond with those of Kandil *et al*., (2012), who claimed that higher salinity level significantly decreased the GSI and germination rate in rice genotypes. Reduced seed germination under salt stress is due to osmotic stress (Nawaz *et al*., 2019). The accumulation of sodium and chloride ions decreases seed germination because they inhibit proper water absorption in salt-stressed plants. In the current study salt stress significantly reduced shoot length as well as root length in all mungbean cultivars. Salt stress inhibited the development of the shoots because it decreased the rigidity of the meristematic tissues, due to the restricted water transport from the rooting region (Hanumantha Rao *et al*., 2016). The transport of a particular ion under salt stress altered the root architecture and anatomy and affected the root growth. According to several earlier studies, plants' adaptation mechanisms for avoiding and reducing salt absorption include a decrease in root length under saline stress (Hasnain *et al*., 2023). However, some genetic alterations also cause the variability in shoot length among cultivars (Zafar *et al*., 2021). Our findings are in accordance with Podder *et al*., (2020), who stated that root and shoot length of mungbean plants was reduced under salt stress. According to Munns *et al*., (2006), the weight of the plant decreased considerably in mungbean cultivars grown under NaCl stress, aligning with our findings. However, the decreased biomass is due to disruption of biochemical and physiological processes as well as the formation of less leaves, which lowers the area of photosynthesis and dry mater (Zafar *et al*., 2015).

Table 3. Dry weight stress tolerance index of mungbean cultivars.

Cultivars	NaCl stress in mM				Rank-ing
	50	75	100	Mean	
NM-2021	82.486	65.75	59.615	69.280a-d	4
NM 121-25	79.746	64.471	73.079	72.4320a-c	3
NM-92	88.137	71.155	63.077	74.1231a-c	3
Abbas Mung	100.863	78.119	68.013	82.33190a	1
NM 19-19	97.443	74.407	71.39	81.0801ab	2
NM-51	70.962	48.123	31.581	50.2221ef	8
NM-54	65.097	31.749	24.054	40.29970f	9
NM-98	64.849	69.916	65.836	66.867b-d	5
NM-2011	71.685	41.006	33.183	48.6246ef	8
Nm-13-1	70.992	50.466	43.459	54.9725d-f	7
NM-2006	80.997	50.221	49.943	60.3870c-e	6
NM-2016	82.643	70.939	49.412	67.6648a-d	4
	79.66a	59.69b	52.7c		

Note: Means with same letter did not vary significantly (p>0.05) in row and column

Table 4. Shoot length stress tolerance index of mungbean cultivars.

Cultivars	NaCl stress in mM				Ranking
	50	75	100	Mean	
NM-2021	74.442	56.564	49.657	60.2209c	5
NM 121-25	70.516	46.918	39.764	52.3992e	7
NM-92	77.981	63.118	55.943	65.6807b	3
Abbas Mung	78.910	64.854	55.658	66.4743ab	2
NM 19-19	80.285	66.097	60.313	68.8988a	1
NM-51	64.377	39.597	31.267	45.0809g	9
NM-54	62.096	29.418	24.261	38.5920h	10
NM-98	75.985	60.328	53.675	63.3296bc	4
NM-2011	61.8955	35.862	25.030	40.9291h	10
Nm-13-1	67.402	44.798	34.940	49.0468f	8
NM-2006	71.595	52.400	43.621	55.8719d	6
NM-2016	75.489	55.909	50.151	60.5164c	5
	71.73a	51.31a	43.68a		

Note: Means with same letter did not vary significantly (p>0.05) in row and column

Table 5. Root length stress tolerance index of mungbean cultivars.

Cultivars	NaCl stress in mM				Ranking
	50	75	100	Mean	
NM-2021	83.259	66.455	57.992	69.23540c	5
NM 121-25	76.005	51.125	38.565	55.2319de	7
NM-92	89.732	76.539	68.874	78.3818ab	2
Abbas Mung	86.249	71.656	65.541	74.4823a-c	3
NM 19-19	90.366	79.703	72.5129	80.86050a	1
NM-51	69.673	37.405	21.575	42.8844fg	9
NM-54	66.059	27.775	11.691	35.17510h	11
NM-98	87.168	71.140	63.619	73.9700bc	4
NM-2011	68.349	30.413	17.441	38.7341gh	10
Nm-13-1	69.963	44.832	31.499	48.7654ef	8
NM-2006	77.838	59.577	46.861	61.4257d	6
NM-2016	72.632	56.232	48.181	59.0153d	6
	71.51a	56.07b	40.44c		

Note: Means with same letter did not vary significantly (p>0.05) in row and column

The physiological and biochemical mechanisms of the plants are hampered by intensity and duration of salinity stress (Munns, 2005; Rozema & Flowers, 2008). Ion toxicity follows osmotic stress, the primary mechanism by which salinity inhibits plant growth (Naher & Alam, 2010). Solute stress causes the root system's water absorption capacity to decrease and accelerates leaf water loss during the early stages and stress due to NaCl aggravates it (Ghosh *et al*., 2015). It is a fact that early plant tolerance mimics tolerance at the maximal level. It has been studied in mungbean (Sen *et al*., 2020), wheat (Laus *et al*., 2021), barley (Nefissi *et al*., 2021), maize (Farooq *et al*., 2015), and rice (Singh & Sengar, 2014). Changes in the early stages of plants affect their yield potential (Tahir *et al*., 2022). Producing cultivars that exhibit variation in how they react to stress in the environment is the primary objective of breeding programs. The current experiment's results demonstrated that stress tolerance indices may be able to characterize certain mechanisms that are indicative of salinity tolerance (Zafar *et al*., 2024).

The stress tolerance indices demonstrate their utility in screening mungbean cultivars in the current study. The cultivar NM19-19 made the maximum score for indices and considered as tolerant variety while the cultivar NM-54 stands at lower rank and proved as sensitive one. These results have resemblances with Zafar *et al*., (2015) and Tahir *et al*., (2022). Therefore, screening of mungbean cultivars showed their genetic potential for salinity tolerance and can be directly grown in saline soil.

The growth of radicles and plumule is reduced under salt stress. The existence of salt in growth medium decreased the water absorption by reduction in osmotic potential that badly affected cell differentiation and division (Negrao *et al*., 2017). Sodium chloride stress considerably decreased the biomass of the plants, radicle and plumule growth, and subsequently whole physiological indices of plant (Ali *et al*., 2022) that could be as a result of ion toxicity and distortion of ion uptake (Sen *et al*., 2020), osmotic effects (Ghosh *et al*., 2015), and water absorption (Laus *et al*., 2021), which lead to a reduction in the synthesis of enzymes and plant hormones (Miransari & Smith, 2019). Zafar *et al*., (2015) determined the reduction in development due to decrease of germination percentage. The salinity stress at germination stage damage the seedlings cell membrane that caused potassium ions to seep out and sodium ions taking the place of calcium (Hasnain *et al*., 2023). Similar results were reported by Podder *et al*., (2020) as well.

Numerous stress tolerance metrics showed substantial relationships with root and shoot length, biomass, and germination. Thus, the germination stress tolerance indices were found to be positively and significantly correlate with each other (Tahir *et al*., 2023). Because of their repeatability and consistency, it is proposed that indices could be a dependable and consistent way to evaluate salt tolerance of cultivars. The correlation related information is very effective for introducing of breeding programs because it gives an opportunity to choose the desirable varieties with required characters (Zafar *et al*., 2015). Many researchers employed different genotypes using cluster analysis to group the traits and observed resemblance among a set of genotypes (Ali *et al*., 2022). The determination of wheat variants given in Zafar *et al*., (2015) also studied the related association in physiological parameters. The study clearly showed that NM-92 is ranked at top by attaining maximum score and is considered a salt tolerant cultivar by attaining a place as cluster 1 in dendrogram (Fig. 1) while, NM-28 is considered as salt sensitive cultivar placed in cluster 3 (Fig. 1).

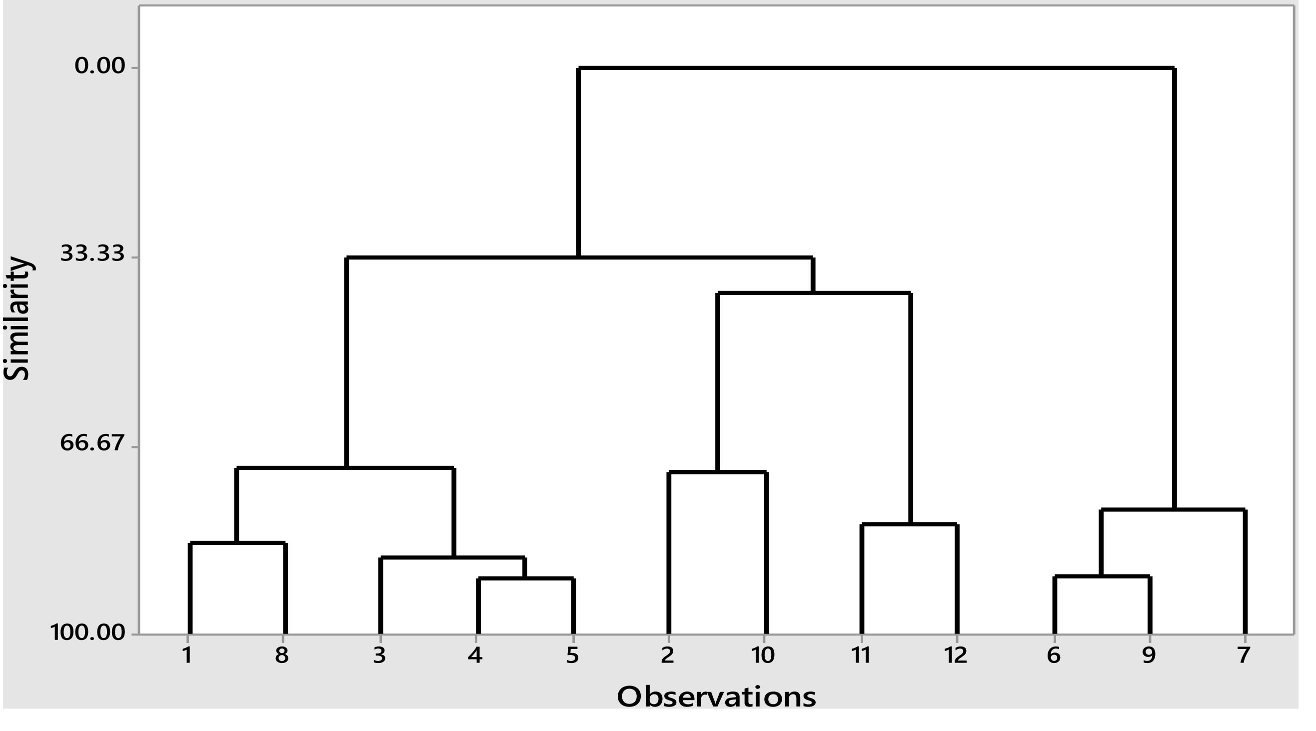

Fig. 1. Dandogram from cluster analysis for salt tolerance in different mungbean cultivars based on physiological indices: a screening tool. Clusters detail; Cluster 1 (Tolerant) (1) NM-2021, (8) NM-98, (3) NM-92, (4) Abbas Mung and (5) NM-19-19 Cluster 2 (Medium Tolerant): (2) NM-121-2, (10) NM-2021, (11) NM-2006 and (12) NM-2016; Cluster 3 (Sensitive): (6) NM-51, (9) NM-2011 and (7) NM-54.

Conclusion

The current findings demonstrated the importance of germination indices in the mungbean germplasm screening process. It was confirmed by cluster analysis, that mungbean cultivars are screened for salt tolerance based on growth parameters. In addition to being utilized to maximize the development of salinity-tolerant mungbean cultivars, the tolerant cultivars can be directly recommended for cultivation in saline soil.

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