THE TOLERANCE OF AN EXTENSIVE COLLECTION OF GARLIC (*ALLIUM SATIVUM* L.) GERMPLASMS TO SALT STRESS – A SUSTAINABLE SOLUTION TO SALT STRESS

AHMAD, J.[1] – WANG, H.[1#] – SONG, J.[1] – NUERAWUTI, M.[1] – ZHANG, X.[1] – YANG, W.[1] – MA, L.[2] – LI, X. X.[1*]

[1]*Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences; Key Laboratory of Biology and Genetics Improvement of Horticultural Crops, Ministry of Agriculture, Beijing 100081, China*
(e-mails: jalil.ahmad1300@gmail.com – J. Ahmad, wanghaiping@caas.cn – H. Wang, songjianping@caas.cn – J. Song, 2780812743@qq.com – M. Nuerawuti, zhangxiaohui01@caas.cn – X. Zhang, wenlongyang@caas.cn – W. Yang)

[2]*Shandong Engineering and Technology Research Center for Garlic, Shandong Jining, 272200, China*
(e-mail: 52569370@qq.com- L.Ma)

[#]*Co-first author*

***Corresponding author**
e-mail: lixixiang@caas.cn

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Abstract. Salinity is a serious problem that limits growth and yield of many agricultural crops worldwide. There is a need for a sustainable long -term solution to this problem. In this study, 354 diverse garlic germplasms were evaluated for their salt -tolerance at the seedling stage by being exposed to NaCl (0.25 mol/L) stress. The salt injury index (SII) along with several plant growth parameters were investigated. The results showed a wide variation of SII from 16.51 to 98.15 among these accessions. All accessions were classified into five groups according to their SIIs, corresponding to different grades of the tolerance to salinity. Among them, two highly tolerant and twenty-four salt-tolerant accessions were screened out. The response of distincit germplasms to salt stress was somewhat different in various trait indices. Moreover, an extremely significantly negative correlation was observed between SII and agronomic traits (PH, LL, LW and RHL) and physiological traits (gs, A, E, Ch). This study provides sustainable solution, original salt-tolerance evaluation technology and valuable materials for the garlic salt-tolerant genetic improvement.
Keywords: *Allium sativum L., NaCl stress, seedling growth, salt injury index, clustering analysis, correlation analysis*

Introduction

Various plants show variable responses to different environmental stresses including abiotic and biotic stresses. Abiotic stresses are a result of non-living components of the ecosystem, such as temperature, drought, waterlogging, salinity, nutrient deficiency, gaseous pollutions, metal toxicity, and so on. Of these, salinity stress is a severe threat to agriculture and food security as one-third of the irrigated land on the earth is affected by salinity (Machado and Serralheiro, 2017). The saline or sodic soils comprise about 6% of the total land all over the world (Munns, 2002). Salinity stress highly declines the crop growth and yield of many field crops (Teshika et al., 2019) and some vegetables, such as garlic, pea, okra, tomato, eggplant, peppers, carrot, cauliflower and potato (Francois, 1994; Shahbaz et al., 2012; Tanveer et al., 2020). Secondary salinization is

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becoming more and more serious in the continuous cropping protected vegetable production (Shahbaz et al., 2012).

Secondary soil salinization is typically caused by an imbalance between transpiration and water inputs from rainfall and irrigation. This imbalance comes in combination with soil characteristics that impede leaching (Cocks, 2001; Mateo-Sagasta and Burke, 2011). Wrong irrigation practices (e.g., waterlogging) and misplanning (e.g., temporal over irrigation) are major drivers of soil salinization (Cocks, 2001; Wichelns and Qadir, 2015)**.** Other factors include the use of unlined canals and reservoirs, and vegetation clearing, in combination with inadequate drainage that filters salts into the groundwaters (Ritzema, 2016)**,** from where the dissolved salts can be remobilized to the upper layers of the soil by means of upward water flows during dry periods (Crescimanno and Garofalo, 2006; Bhutta and Smedema, 2007)**.** The quality of the irrigation water and rational application of fertilizer are also very important to avoid secondary salinization.

Salinity excess affects plant growth through many aspects of physiological and biochemical processes in the plant. Saline stress results in osmotic pressure and ionic stress, which impair the pivotal cellular function. Osmotic stress minimizes the water availability, causes dehydration and stomatal closure and slows down the rate of biochemical reactions (Acosta et al., 2017; Munns, 2002). Salinity stress alters some of the physiological process of plants, such as respiration rate, mineral distribution, membrane stability and turgor pressure (Hasegawa, 2013; Garrote et al., 2015). Shoot growth and early flowering are also influenced by salinity due to the inhibitory effect of salt in growing points on cell division and cell enlargement (Alom et al., 2016).

Plants withstand the salt stress by several mechanisms including high complexity or low complexity mechanisms. The former one is thought to be involved in alterations of many biochemical pathways. The later one is believed to induce coordination for the preservation of complex processes (Nasri et al., 2015). The high-complexity mechanism protects the main processes such as respiration and photosynthesis and retains important features such as plasma membrane interactions, cytoskeleton, cell wall (Gupta and Huang, 2014) and chromatin structural changes such as polyploidization, DNA methylation, DNA removal or amplification of unique sequences (Walbot and Cullis, 1985). Anyway, the genetic mechanism may be the major controller behind the different mechanisms.

Economic reasons or scarcity of freshwater are the major limitations to the soil salinization recovery in many situations. The only possible feasibility is the development of salinity tolerant varieties (Hoque et al., 2015), which could be developed through the germplasm selection and genetic improvement. Still, the success is related to the extent of genetic variation of tolerance to salinity among available germplasm for a crop species. Simultaneously the complexity of traits, limited knowledge about physiology and genetics of tolerance related attributes, and shortage of efficient selection domain are the major constrains to salinity tolerance breeding programs. What is more, optimizing saline conditions in the field and greenhouse for identification could be temporary and expensive.

Genetic characterization of useful germplasm is the first step towards releasing tolerant cultivars. Many researchers have demonstrated that evaluating the salt tolerance at the vegetative stage of a plant species is important to determine the ultimate tolerance of the species (Aslam et al., 1993; Uddin and Hossain, 2018; Kakar et al., 2019; Sikder et al., 2020). The early vegetative stage of a crop is regarded as the most dangerous stage, where plant yield is determined by (Uddin and Hossain, 2018) reported that

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selection of a crop is noted to be important at the vegetative stage for at least two reasons. Firstly, under controlled conditions in limited space, vegetative growth rates can be calculated easily and relatively in a short time (4-6 weeks). Secondly, under saline conditions, rapid vegetative development reflects a plant response to the stress environment and its capacity to produce additional resources for growth. The difference in salinity tolerance at vegetative stage has been reported in vegetables crops (Blum, 2018). Furthermore, a more significant yield loss has been reported when plants were exposed to salinity at the early growth stage than the divulgence at a later part of growing (Machado and Serralheiro, 2017).

A few researches have been done on salt tolerance of garlic. Francois (1994) reported that yield components (bulb weight and diameter) were reduced with increasing salinity, as well as the percent of solids which is a major component of bulb quality. Shoot dry weight was less susceptible to salinity stress than bulb weight, but higher concentration of chlorine, sodium and calcium accumulated in leaf tissues than in bulb by a small-scale experiment. Saline water irrigation significantly decreased the number of leaves and plant height of garlic (Shama et al., 2016).

Therefore, the main objective of this study was to evaluate the salt-tolerance of garlic germplasm at the seedling stage based on agronomic characteristics in a pot culture system on a large scale for the first time and understand the genetic variation of salinity tolerance and the relationship between the salt-tolerance and the agronomic/physiological characteristics for garlic genetic improvement of salt-tolerance and effective utilization of salt-tolerant garlic varieties in saline soil exploitation.

Materials and methods

The garlic germplasm of 354 accessions collected from in 15 province of China and 30 other countries were conserved in the national field gene bank of vegetative propagation vegetables on Lang fang farm of the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences in the suburb of Beijing, China. The experiment was conducted in a solar greenhouse using a pot culture method during early Spring and were arranged according to a completely randomized block design for each accession under stress and control with three replicates. Ten garlic cloves for each replicate were sowed in plastic pots (8 × 8 cm) containing peat and vermiculite (1:1). Hoagland's solution was used as a source of nutrient. Each pot with garlic cloves was irrigated with irrigation water in the first week and with the 1/2 fold Hoagland's solution prepared with irrigation water during the second week and the third week after sowing once a week. Each pot for salt stress was subjected to 1.5% concentration of NaCl (0.25 mol/L) prepared with irrigation water starting from the beginning of the fourth week after sowing, continuously four weeks, once in each week. In contrast to salt treatment, each pot under the control was irrigated with irrigation water at the same frequency.

Investigation of salt injury grades and calculation of salt injury index

Visual salt injury grades for each plant in different accessions was determined after one week after finishing the salt treatment according to the following standard we modified based on the method by IRRI (2014) (*Table 1*).

Eight plants per replicate for each accession were scored. Salt injury index (SII) were calculated by the following formula (*Eq. 1*):

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$$SII = \frac{\sum (s \times n)}{N \times s} \times 100 \qquad \text{(Eq.1)}$$

where: ∑ = The sum of the product of each grade value and the number of plants at each grade; s = the value of each grade; n = the number of plants at each grade; N = the total number of plants investigated; S = The highest value of injury grade.

Salt tolerance was classified according to clustering analysis result based on the average SII value of three replicates for each accession.

Table 1. *Modified standard of visual salt injury of each plant at the seedling stage*

Grade	Observation of visual salt injury (VSI)
1	Plant grows normally, and only the leaf tips of 2 leaves, or less than 2 leaves of a plant are yellowing
3	Plant grows basically normal, and the leaf tips of 3~ 4 leaves are yellowing or curly slightly
5	Plant growth is retarded partly; 5 ~ 6 leaves yellowing or curly
7	Plant growth is severely retarded or even ceases; 6 ~ 7 leaves of a plant are yellowing or becoming dry
9	More than 7 leaves are yellowing and severe, the whole plants are dead or dying

Measurement of agronomic traits

Plant height (PH), leaf length (LL), leaf width (LW), total leaves number and healthy leaves number of 354 accessions were measured individually in the eighth week after sowing. The ratio of healthy leaves number to total leaves number per plant was calculated by the formula (*Eq. 2*):

$$RHL = \frac{Healthy\ leaves\ number}{Total\ leaves\ number} \times 100 \qquad \text{(Eq.2)}$$

In order to understand the effect of salt stress on plant growth change, the relative value of plant height (RPH), leaf length (RLL), leaf width (RLW) and ratio of healthy leaves (RRHL) under salt stress and under control were calculated referred to the following formula (*Eq. 3*):

$$RPH = \frac{Plant\ height\ under\ stress}{Plant\ height\ under\ control} \qquad \text{(Eq.3)}$$

Measurement of physiological indexes

Sub-stomatal CO_2 concentration (Ci, μmol mol^{-1}), assimilation/respiration (A, μmol CO_2 m^{-2} S^{-1}), stomatal conductance (gs, mmol H_2O m^{-2} S^{-1}), transpiration (E, mmol H_2O m^{-2} S^{-1}) of 81 accessions selected randomly from 354 accession as representatives was determined by CIRAS-3 portable photosynthesis system (PP system, Amsbury, MA, USA) after seven weeks of sowing in order to shorten measurement time and minimize experiment error. Data were automatically recorded by the CIRAS-3 every 5 s. The CO_2 concentration (380 μ mol mol^{-1}), relative humidity (60%), and leaf temperature 28 °C were maintained using an automatic control device on the CIRAS-3. Red- blue light (90%: 10%) was provided by the LED light unit in the CIRAS3.

The Soil and Plant Analyzer Development (SPAD) values can present the relative content of chlorophyll and be measured by the portable SPAD-502m emitting red light (650 nm) and infrared light (940 nm) via transmission. By the difference of optical

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density between the two wavelengths, the relative content of chlorophyll (SPAD value) can be obtained. For the measurement, three plants for each replicate in each accession and three leaves in the middle of each plant were selected, and each measurement was repeated three times.

Statistical analysis

Statistical analysis of basic data is carried out in Excel 365. Cluster analysis was performed based on the unweighted pair-group method with arithmetic means (UPGMA), using the SPSS for windows. The correlation analysis among the index values of these traits under stress was performed using Pearson’s Correlation Coefficient using R studio software version 1.2.500.1.

Results

Variation in salinity tolerance of garlic germplasm from salt injury observation

Based on the performance of 354 garlic germplasm under salt stress, the SII of all tested germplasm under salt stress were calculated and distributed from 16.51 to 98.15. A cluster analysis based on the un-weighted pair group method assigned the 354 germplasm into five main groups. Group I represents two accessions of 8N327 and 8N825, recognized as highly tolerance to salt stress with SII range from 16.51 to 17.59. Group II exhibits 24 germplasms, having the SII from 21.83 to 34.72 regarded as tolerance to salt stress. Group III illustrates 269 germplasm of moderately tolerance, which SII distributed from 35.45 to 57.01. Group IV covers 54 accessions recognized as susceptible to salt stress having the SII from 57.41 to 81.48. Group V include 5 accessions with SII range of 91.53 to 98.15 known as highly susceptible to salt stress (*Table 2; Figs. 1* and *2; Table A1* in the *Appendix*).

Table 2. *Classification of the germplasms on the base of SII*

Tolerance	Germplasm name	No of germplasm
Highly tolerant	8N327 8N825	2
Tolerant	8N850 8N847 8N724 8N869 8N908 8N911 8N325 8N360 8N032 8N128 T-167 8N167 8N364 T-141 8N141A 8N141B T-261 8N038A 8N719 8N261 T-17 T-258 8N026B 8N748	24
Sensitive	8N422 8N503 8N406 8N312 ZS-9 8N556 8N423 8N587 8N002 8N764 8N512 8N273 8N421 8N560 8N930 8N514 JX-1 8N372 8N076 8N566 8N654 8N786 WQS 8N780 8N629 8N037 JX-4 8N002B 8N410 8N728 8N249 8N975 8N254A 8N761 8N218 ZSS 8N324 8N013 8N529 8N427 8N332 8N561 8N675 8N526 8N950 8N030A 8N1040 8N545 8N760 8N559B 8N1046 8N970 8N676 8N1042	54
Highly sensitive	8N952 8N649 8N953 8N972 8N954	5

Agronomic traits response of different garlic germplasm to salt stress

NaCl salt stress decreased the growth performance of seedlings in most of 354 garlic germplasm to a different extent. The plant height showed a dispersing and great variation in the growth reduction of most germplasm under stress as compared to the control. Similarly, larger decreases in leaf length were observed under stress for most accessions. The leaf width and the ratio of healthy leaves number of germplasms also displayed a certain degree of difference between salt treatment and the control (*Fig. 3*). In more details, RPH varied from 0.42 to 1.00 with a mean of 0.73 and the frequencies

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of germplasm resources with different RPH values were almost normally distributed. Some germplasm such as 8N327 (0.87), 8N570 (0.93), 8N654 (0.94), 8N738 (0.95) T-167 (0.96) 8N222 (0.97) and T-17 (1.00) had the RPH value over 0.9, indicating these germplasms were more salt tolerant than other germplasm based on plant height reduction. Lower RPHs was found in 8N830 (0.47), 8N808 (0.44), 8N675 (0.44), etc., indicating these germplasms were highly susceptible to salt stress based on plant height reduction (*Fig. 4a*). RLL values ranged between 0.41 and 0.97 with a mean of 0.64 and the frequencies of germplasm resources with different RLL values were also nearly normally distributed. T-258 (0.89), 8N424(0.91), 8N239 (0.92), 8N992 (0.96) and 8N069 (0.97) had higher RLL values, indicating that they were salt-tolerant based on leaf length change; whereas, 8N586(0.45), 8N888 (0.44), MS No1 (0.44) 8N643 (0.43), and 8N1008(0.43) were highly susceptible to salt stress (*Fig. 4b*). RLW values varied from 0.43 to 1.00 with a mean of 0.86 and the frequency distribution of germplasm resources with different RLW values was skewed, indicating the leaf width of most accessions was relatively stable under salt stress. Higher relative values of leaf width were recorded for T-17 (0.86), 8N826 (0.87), 8N032 (0.93), 8N325 (0.96), T-141 (0.97) 8N847 (0.99), 8N519 (0.97), and 8N078B (1.00). Lower RLWs were found for 8N780 (0.43), 8N953 (0.49), 8N1005 (0.50) and 8N1022 (0.50) (*Fig. 4c*). RRHL values ranged between 0.71 and 0.99, within a mean of 0.88 and the frequencies of germplasm resources with different RRHL values were normally distributed. 8N758 (0.91), 8N249, (0.92), 8N876 (0.93), 8N808 (0.94), 8N066 (0.95), 8N922 (0.96), 8N1005 (0.97), 8N1004 (0.98), 8N126 (0.99) had the higher RRHL, indicating that these germplasms were more tolerant to salt based on RHL reduction than other germplasm. Lower RRHLs values were found for 8N586 (0.71), 8N675 (0.75) and 8N676 (0.75), showing that these germplasms are more susceptible to salt stress based on the RHL reduction (*Fig. 4d*). From the results above, it is obvious that the seedling growth of most accessions was decreased to different extent in the four parameters. However, the tolerance performance of different germplasm affected by salt stress is different in various agronomical traits. The SII for each accession is a comprehensive index, which is contributed by different single traits in different ways.

Correlation among agronomic and physiological traits affected by salt stress

By using 81 accessions as a pilot study, correlation analysis exhibited the association among different agronomic traits and physiological traits of garlic germplasm (*Fig. 5*). SII as an important comprehensive agronomic trait is extremely significantly negative correlated with PH (r = -0.65), LL (r = -0.64), LW (r = -0.53), RHL (r = -0.53), gs (r = -0.29), A (r = -0.44) and E(r = -0.60) at $P_{0.001}$, highly significant with Ch (r = -0.20) at $P_{0.05}$. Among other morphological and physiological traits, PH is extremely significant positive correlated with LL (r = 0.76), LW (r = 0.66), RHL (r = 0.47), A (r = 0.42), E (r = 0.50) at $P_{0.001}$ and highly significant positive with gs (r = 0.25) at $P_{0.05}$. LL is extremely significantly positive related with PH, LW, RHL, A, E and Ch at $P_{0.001}$. LW is highly significantly positive with RHL, A and E at $P_{0.05}$ except for the relationship with PH and LL. RHL is also extremely significantly positive related with A (r = 0.50), E (r = 0.64) and Ch (r = 0.48). Ci is extremely significantly positive with gs (r = 0.41), A (r = 0.34) and E (r = 0.34). gs is extremely significantly positive with A (r = 0.35), E(r = 0.26) and Ci. A is extremely significantly positive with E(r = 0.65), Ch (r = 0.27) besides PH, LL, RHL, Ci and gs. E is extremely significantly positive with Ch (r = 0.37) LL.RHL and A.

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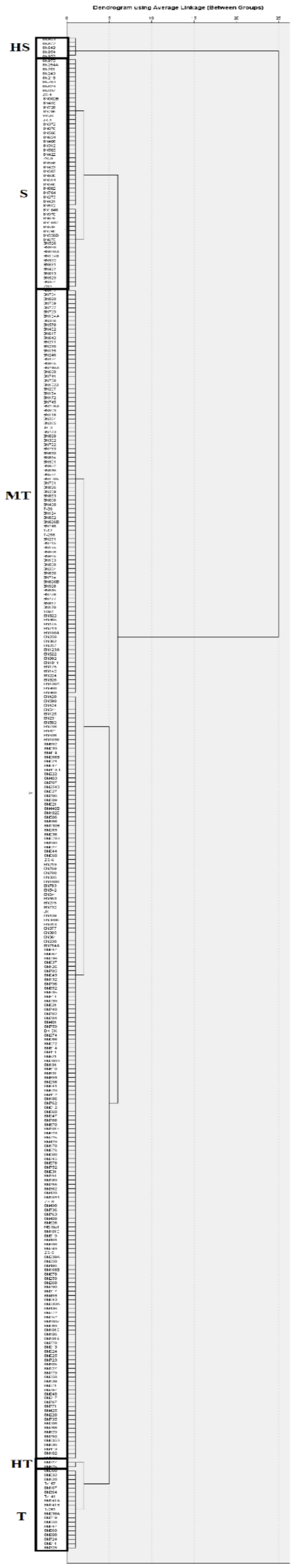

Figure 1*. Cluster analysis of the garlic germplasm based on the salt injury index (SII) using unweighted pair-group method (UPGMA)*

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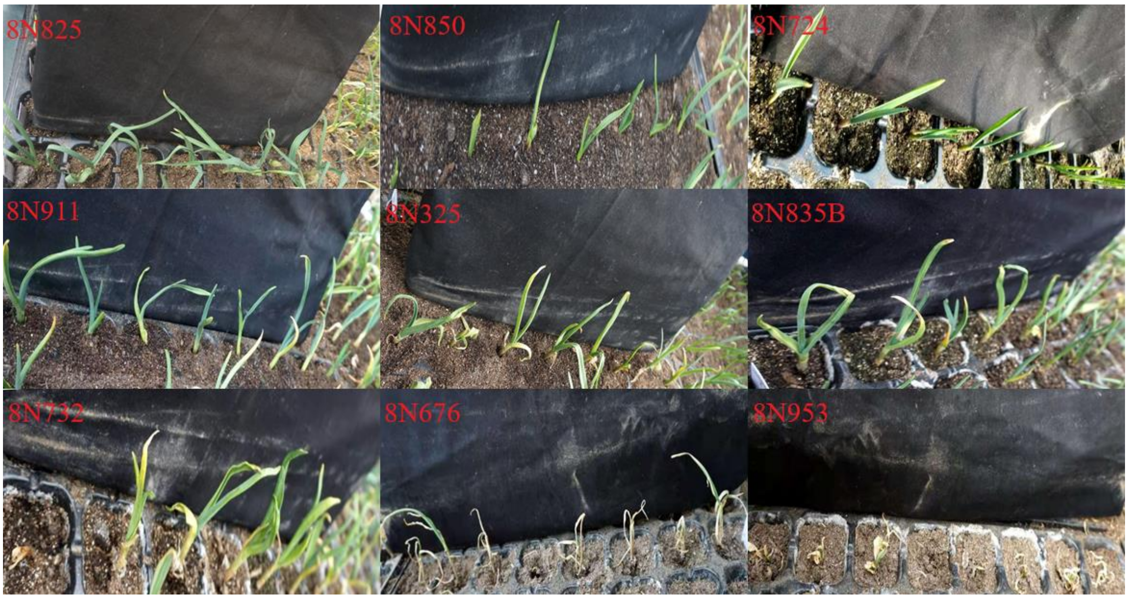

Figure 2. *Growth performance of representative germplasm with different tolerance to salt stress*

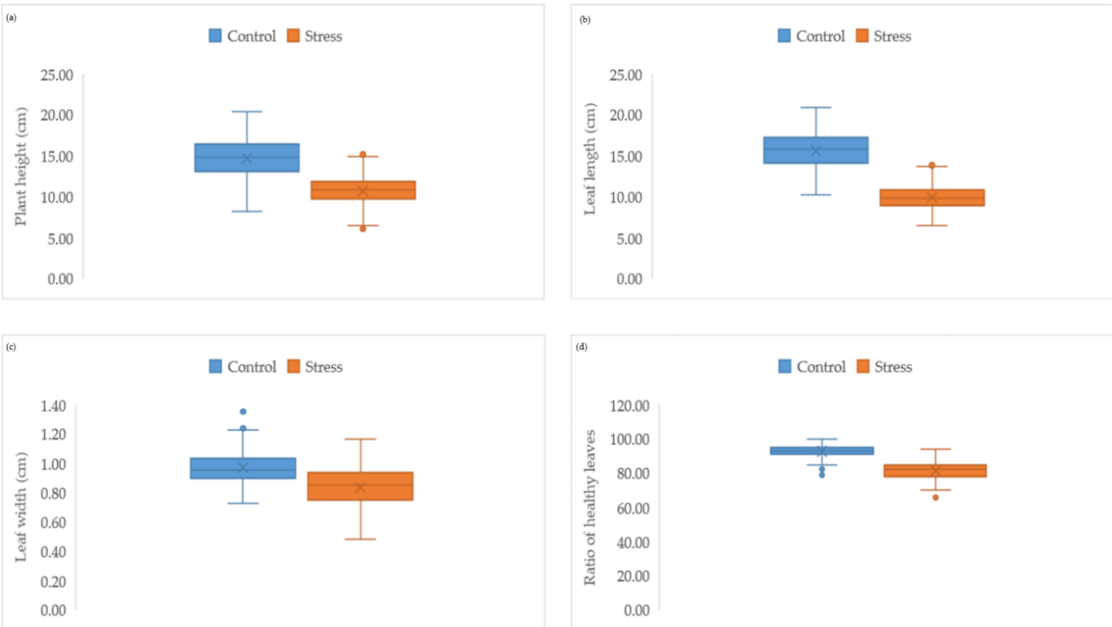

Figure 3. *The value distribution and comparison of agronomic traits of all garlic germplasm under salt stress and under control. (a) Plant height (cm); (b) leaf length (cm); (c) leaf width (cm); (d) ratio of healthy leaves*

Discussion

Tolerance to salt is important target trait for garlic breeding. Reasonable identification method is the basis of successful salt tolerance evaluation of garlic germplasm or breeding materials. Mass screening for salt tolerance of crops is complex, even in the soil culture or directly in the field because of multiplicity limitations like the

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status of soil fertility, irrigation management (Perez-Harguindeguy et al., 2016), salinity type (Munns and Tester, 2008) meteorological aspects like temperature and humidity) and as well as natural variation in fields (Hasana and Miyake, 2017). Field screening techniques have been reported confronting the main problem of soil heterogeneity, and a limited number of genotypes could be handled (Aslam et al., 1993).

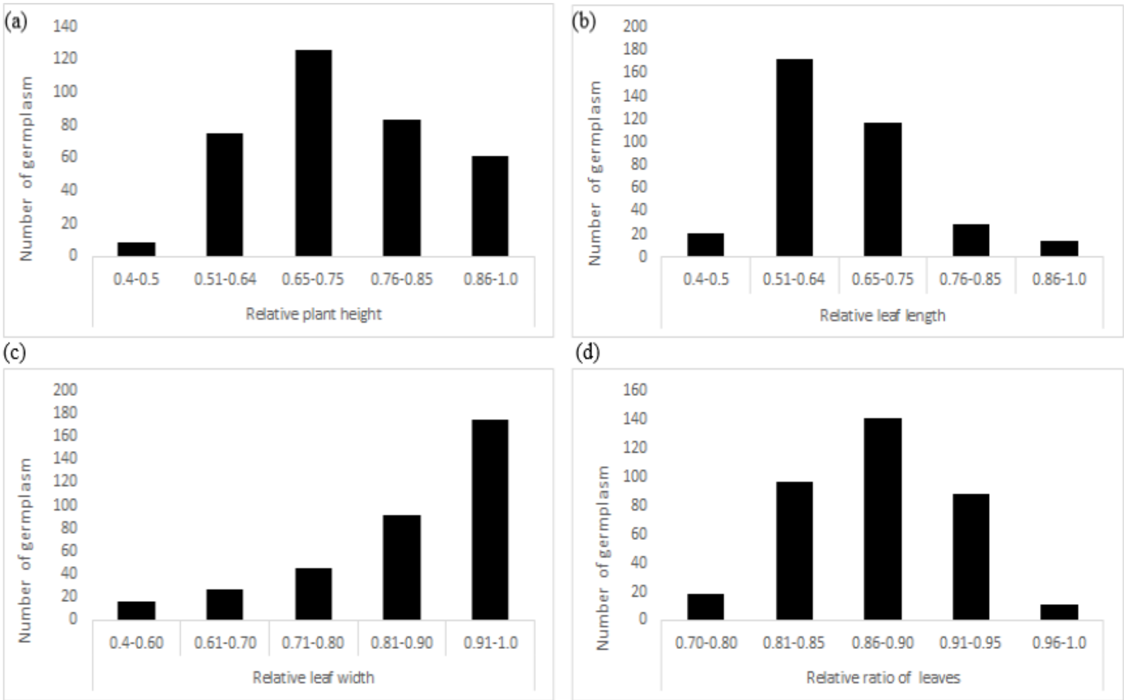

Figure 4. *Germplasm frequency distribution in different relative value range of each growth parameters under salt stress to under control. (a) Relative plant height; (b) relative leaf length; (c) relative leaf width; (d) relative ratio of leaves*

In the present study, we designed a pot culture method to characterize saline tolerance of garlic germplasm at the seedling stage. We screened out two highly tolerant and 24 tolerant garlic germplasm which are valuable for breeding and further study. The SII from visual salt injury observation is a comprehensive performance of plants under salt stress. It is reflected in many aspects of plant growth. The method is rapid and economical, and can easily meet the experimental conditions with the following advantages: (i) the salinity is standardized in the pot, or among pots, (ii) the irrigations quantity could be controlled, (iii) problems associated with salt depletion have been overcome. The effectiveness of this method was also confirmed by previous study in other crops such as rice (Kakar et al., 2019) and cotton (Sikder et al., 2020).

A reliable technical standard and index system for salt injury grading and tolerance classification is also a key to successfully develop a target-specific variety for salt tolerance. Visual symptoms of salt stress are mainly chlorosis of leaves, leaf tips burning, plant stunted growth and wilting (IRRI, 2014), and visual salt injury grades in rice. Sabra et al. (2012) studied the salt injury in three different Echinacea species by observing the appearance of leaves to develop a five-point scale according to the severity of necrotic tissues and number of injured plants. In the present study, we successfully distinguished garlic germplasm with the extensively distribution of SII

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value from 16.51 to 98.15 based on the modified grading standard of visual salt injury of leaves and plants in garlic (*Table 1*). For the salt tolerance evaluation, we did not use directly the arbitrary man-made resistance grading standard as in previous studies (Kopittke et al., 2009; Bolton and Simon, 2019). By the aid of cluster analysis based on the SII of each germplasm, we divided all germplasm into five groups, which exhibits high homogeneity within a cluster and high heterogeneity between clusters and help to setup a reasonable salt tolerance classification system according with the actual tolerance distribution in garlic and some other crop (Pradheeban et al., 2015).

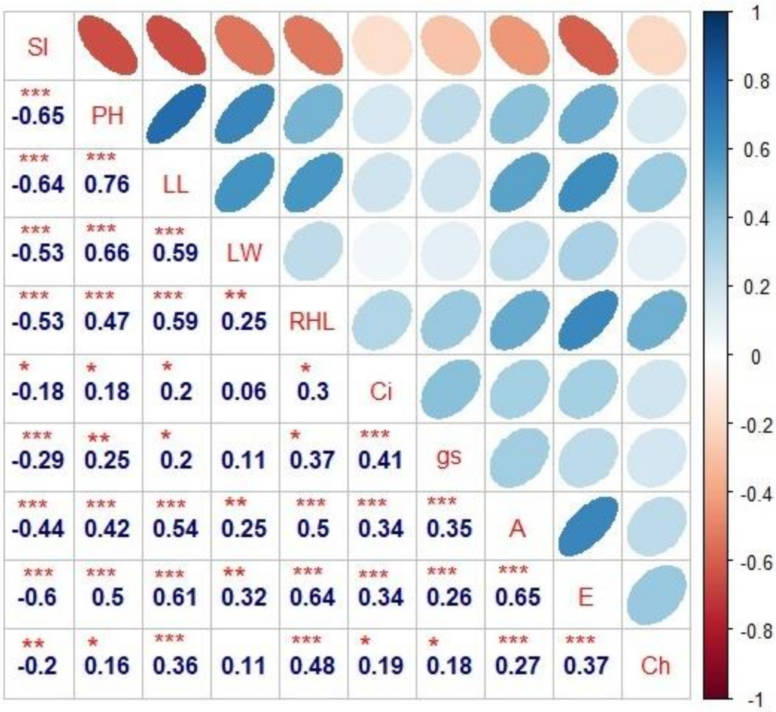

***Figure 5.** Correlation coefficients among the growth attributes of garlic. The upper diagonal represents the correlation coefficient with significance levels at *p < 0.05, **p < 0.01, and *** p < 0.001, respectively; PH (plant height), LL (leaf length), LW (leaf width), RHL (Ratio of healthy leaves) Ci (Substomatal CO_2 concentration), gs (Stomatal Conductance), A (Respiration), E (Transpiration), Ch (Chlorophyll content)*

Salt stress reduces plant growth and productivity by affecting morphological, anatomical, biochemical and physiological characteristics, processes and functions. Reduced plant height and other morphological characters are the most distinct and obvious effect of salt stress. Depressed growth due to salinity is attributed to several factors such as, water stress specific ion toxicity and ion imbalance stress or induced nutritional deficiency. Our findings show that, plant height of all the germplasms deceased by the salinity stress. The reduced plant height might be attributed to the direct effect of excess salt on plant tissues and poor intake of minerals. Reduced plant height under saline conditions has been observed in garlic (Shama et al., 2016) and quinoa (Cai and Gao, 2020).

Leaf area represents the plant growth measurement (leaf length and width), which can be affected by salt stress. Our results showed a decrease in leaf length and width with NaCl stress. These results agree with Mathur et al. (2006); they reported that the

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moth bean plant (*Vigna aconitifolia* L.) with increasing concentration of sodium chloride, led to a decrease in leaf area. This reduction was inversely proportional to the concentration of NaCl. Also, a significant decrease in leaf area of sugar cane (*Beta vulgaris* L.) in response to salt stress using concentration zero, 50, 100, 150 mmol of sodium chloride, has been reported (Jamil et al., 2007). Other supporting results include those of Zhao et al. (2007), with their study on oat (*Avena sativa* L.) Yilmaz and Kina (2008), with their study on *Fragaria x anassa* (L.).

NaCl salt stress decrease ratio of healthy number of leaves in plants, compared with the control plant. The results have been confirmed by the results of Karen et al. (2002), with their study on *Cirer arietinum* L. and Lopez et al. (2003), with their study on the teprary bean (*Phaseolus acutifolius* L.), cowpea (*Vigna unguiculata* L.), and wild bean (*Phaseolus filiformis* L). They mentioned that, the treatment of NaCl reduced the number of leaves compared with control plants. Shama et al. (2016) reported that saline water irrigation significantly decreased the number of leaves and plant height of garlic. The decrease of leaves number may be due to the accumulation of NaCl in the cell wall and cytoplasm of the leaves. At the same time, their vacuole sap cannot accumulate more salts and, thereby decreases the concentration of salt inside the cells, which ultimately leads to their quick death (Munns, 2002).

Salinity tolerance has been measured based on both the absolute and relative values of plant growth, which are very important in assessing plant material of diverse origin (Ashraf and Waheed, 1990; Ding et al., 2018). The 354 accessions here were assessed for their ability to sustain growth under saline conditions, as absolute values under stress and relative values compared with control. These results were in line with other previous studies that salinity suppressed agronomic traits in *Triticum durum* Desf (Noori and McNeilly, 2000) and sweet sorghum (Ding et al., 2018).

From the correlation analysis, although some correlation coefficients were not very high, they were significant. This is mainly because of the large-scale samples, some of which may contribute interference to the final result. The reliability of the weaker correlation results from large-scale at an enhanced significance criterion of $P_{0.001}$ besides $P_{0.01}$ *and* $P_{0.05}$ are equally recognized as the strong correlation in small samples (Abdelghany et al., 2020; Azam et al., 2021). SII as a comprehensive index are involved in many aspects of plants which may be affected and interacted with each other. In our study, among indexes of the observed agronomic traits at seedling stage, it was found that SII was extremely significantly negative correlated with all the measured agronomic traits under stress, and that there was also extremely significantly negative correlation between the physiological traits affected by salt stress, which provides important clue to assess the plant response to salt stress. These findings are consistent with the previous study in pistachio (Karimi and Roosta, 2014).

NaCl stress can decrease in physiological activities and consequently hinder the photosynthetic mechanism of the plant (Khan, 2016). In the current study, NaCl stress is a negative correlation with physiological traits such as sub-stomatal CO_2 concentration (ci), stomatal conductance (gs), transpiration (A), respiration (E), and relative chlorophyll content (Ch). Generally, CO_2 exchange was regarded as an important indicator of the growth of plant, because of its direct link to net productivity (Asharf, 2004). It was proven that sub-stomatal CO_2, stomatal conductance, transpiration and rate of photosynthesis of all the parameters are affected by salt stress. These results agree with previously report in rocket (*Eruca sativa* (L.) *Mill.*), where there were negative correlation between salinity and gas exchange parameters (Hniličková et al.,

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2017). The decrease in gaseous exchange attributes in the current study might be associated with salinity-induced osmotic stress that rendered the growing plants out of the water and hampered the rate of transpiration, which further excavated water and CO_2 supply for normal photosynthesis (Shahzad et al., 2019). They might also be due to osmotic and hormonal imbalances created by the generation of reactive oxygen species (ROS) in plant cells that impaired carbohydrates metabolism and hence the photosynthetic efficiency (Bergmann et al., 2008).

The impact of photosynthesis can be evaluated from the photosynthetic pigments. With increasing salt stress, the SPAD value decreased. Previous studies have described that salinity stress declined photosynthetic pigments of plants (Anand and Byju, 2008) and have also shown that the SPAD value measured by the SPAD chlorophyll meter is highly correlated with chlorophyll content (Anand and Byju, 2008). In the present study the chlorophyll content are negative correlation with salt stress. It was reported that the effect of salt stress on chlorophyll was varied species to species, and some studies have shown that salt stress can inhibit the chlorophyll synthesis of plants (Wang et al., 2015). Heidari (2012) reported that salt stress is a negative correlation with chlorophyll content in *ocium basilicum* (L.). Gouveianeto et al. (2011) found the main reason for the decrease in chlorophyll content caused by high salt concentration was the blocking of electron transport.

Conclusions

This study revealed a widely variation of salt tolerance during the seedling stage in a collection of diverse garlic germplasms based on an improved identification method and evaluation system. The discovery of salt tolerant accessions could not only serve as potential materials for identification of salt tolerant associated QTLs/genes, but also were promising for breeders to develop salt-tolerant cultivars. Although the respective agronomic traits of different germplasm had somewhat different response to salt stress, there were significantly or extremely significantly positive correlation among the most concerned agronomic and physiological traits affected by salt stress and the significantly or extremely significantly negative relationship between SII and morphological traits (PH, LL, LW, RHL), also between SII and physiological traits (gs, A, E, Ch) under stress. All these results provide the valuable base for the establishment of the reasonable salt-tolerance identification and classification standards and technical index, and further salt tolerance mechanism study.

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APPENDIX

Table A1. *Germplasms origin and their classification on the base of salt tolerance*

Sr. No	Germplasms ID	SII	Group	Tolerance	Province/world
1.	8N327	16.51	1	HT	Yunnan Province
2.	8N825	17.59	1	HT	Tajikistan
3.	8N850	21.83	2	T	Washington, United States
4.	8N847	22.84	2	T	Washington, United States
5.	8N724	25.00	2	T	Pakistan
6.	8N869	25.28	2	T	Uzbekistan
7.	8N908	25.40	2	T	Washington, United States
8.	8N911	25.74	2	T	Washington, United States
9.	8N325	26.46	2	T	Yunnan Province
10.	8N360	28.70	2	T	Europe
11.	8N032	28.70	2	T	Shanghai
12.	8N128	29.31	2	T	Jiangsu Province
13.	T-167	29.38	2	T	Yunnan Province
14.	8N167	29.78	2	T	Yunnan Province
15.	8N364	29.89	2	T	Korea
16.	T-141	30.56	2	T	Shandong Province
17.	8N141A	30.89	2	T	Shandong Province
18.	8N141B	31.48	2	T	Shandong Province
19.	T-261	32.25	2	T	Jiangsu Province
20.	8N038A	32.30	2	T	Shandong Province
21.	8N719	33.33	2	T	Former Serbia and Montenegro
22.	8N261	34.04	2	T	Jiangsu Province
23.	T-17	34.12	2	T	Jiangsu Province
24.	T-258	34.14	2	T	Jiangsu Province
25.	8N026B	34.72	2	T	Washington United States
26.	8N748	34.72	2	T	Turkey
27.	8N715	35.45	3	MT	Illinois, United States
28.	8N195	35.66	3	MT	Jiangsu Province
29.	8N183	36.11	3	MT	Hubei Province
30.	8N899	36.11	3	MT	Washington United States
31.	8N655	36.11	3	MT	North Macedonia
32.	8N808	36.42	3	MT	Washington United States
33.	8N826B	36.90	3	MT	Washington, United State
34.	8N928	37.04	3	MT	Viet Nam
35.	8N234	37.17	3	MT	Hubei Province
36.	8N830	37.19	3	MT	Washington, United State
37.	8N714	37.30	3	MT	Washington, United States
38.	8N822	37.57	3	MT	Sachsen-Anhalt, Germany
39.	109Z	37.66	3	MT	Jiangsu Province
40.	8N777	37.70	3	MT	Andalucía, Spain
41.	8N817	37.70	3	MT	Slovenia
42.	8N170	37.74	3	MT	Yunnan Province

43.	8N865	37.96	3	MT	Bulgaria
44.	8N145	38.10	3	MT	Sichuan Province
45.	8N845	38.40	3	MT	Washington, United State
46.	8N776	38.43	3	MT	Andalucía, Spain
47.	8N713	38.89	3	MT	California, United States
48.	8N1004	38.89	3	MT	Jiangsu Province
49.	8N992	39.05	3	MT	Jiangsu Province
50.	8N123A	39.15	3	MT	Jiangsu Province
51.	8N622	39.15	3	MT	Shandong Province
52.	8N257	39.29	3	MT	Jiangsu Province
53.	8N258	39.33	3	MT	Jiangsu Province
54.	8N862	39.35	3	MT	Jiangsu Province
55.	8N860	39.68	3	MT	Korea, South
56.	8N130A	39.81	3	MT	Anhui Province
57.	8N900	39.90	3	MT	Washington United States
58.	8N326	40.11	3	MT	Yunnan Province
59.	8N1011	40.21	3	MT	Jiangsu Province
60.	8N175	40.21	3	MT	Yunnan Province
61.	8N142	40.28	3	MT	Shandong Province
62.	8N224	40.33	3	MT	Shaanxi Province
63.	8N1005	40.74	3	MT	Jiangsu Province
64.	8N209	40.74	3	MT	Liaoning Province
65.	8N781	40.74	3	MT	Washington United States
66.	8N826	40.74	3	MT	Washington, United State
67.	8N846	40.74	3	MT	Washington, United State
68.	8N534	40.74	3	MT	Guizhou
69.	8N863	40.81	3	MT	Italy
70.	8N832	41.01	3	MT	Washington, United State
71.	T-36	41.08	3	MT	Shandong Province
72.	8N124	41.09	3	MT	Jiangsu Province
73.	8N096	41.14	3	MT	Gansu Province
74.	8N490	41.14	3	MT	United States
75.	8N922	41.42	3	MT	Yunnan Province
76.	8N864	41.62	3	MT	Bulgaria
77.	8N302	41.67	3	MT	Yunnan Province
78.	8N660	41.67	3	MT	Turkey
79.	8N722	41.67	3	MT	Chile
80.	8N753	41.67	3	MT	Washington United States
81.	8N773	41.67	3	MT	Andalucía, Spain
82.	8N890	41.67	3	MT	Washington United States
83.	8N501	41.85	3	MT	Egypt
84.	8N741	42.06	3	MT	Washington United States
85.	8N758	42.06	3	MT	Castilla-La Mancha, Spain
86.	8N154	42.15	3	MT	Shandong Province
87.	8N1022	42.20	3	MT	Shandong Province
88.	8N207	42.20	3	MT	Hebei Province

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89.	8N240A	42.33	3	MT	Jiangsu Province
90.	8N668	42.33	3	MT	Brazil
91.	8N365	42.55	3	MT	Korea
92.	8N118	42.59	3	MT	Shaanxi Province
93.	8N264	42.59	3	MT	Shandong Province
94.	8N206A	42.59	3	MT	Hebei Province
95.	8N903	42.59	3	MT	Washington United States
96.	8N172	42.68	3	MT	Yunnan Province
97.	8N745	42.72	3	MT	Albania
98.	JX-3	42.86	3	MT	Shandong Province
99.	8N898	43.06	3	MT	Washington, United States
100.	8N507	43.12	3	MT	Jiangsu
101.	8N734	43.12	3	MT	Andalucía, Spain
102.	8N789	43.21	3	MT	Varna, Bulgaria
103.	8N570	43.34	3	MT	Washington, United States
104.	8N104A	43.52	3	MT	Shaanxi Province
105.	8N366	43.52	3	MT	Korea
106.	8N737	43.52	3	MT	Shumen, Bulgaria
107.	8N799	43.52	3	MT	USA
108.	8N855	43.78	3	MT	Washington, United States
109.	8N246	43.92	3	MT	Yunnan Province
110.	8N434	43.98	3	MT	Henan
111.	8N155	44.09	3	MT	Shandong Province
112.	8N211	44.14	3	MT	Ningxia
113.	8N260	44.14	3	MT	Jiangsu Province
114.	8N642	44.44	3	MT	Guizhou
115.	8N402	44.44	3	MT	Hebei Province
116.	8N617	44.44	3	MT	Shandong Province
117.	8N252	44.84	3	MT	Yunnan Province
118.	8N436	44.91	3	MT	Henan
119.	8N772	44.97	3	MT	Andalucía, Spain
120.	8N1007	45.22	3	MT	Shandong Province
121.	8N069	45.24	3	MT	Shaanxi Province
122.	8N1019	45.37	3	MT	Shandong Province
123.	8N778	45.37	3	MT	Andalucía, Spain
124.	8N913	45.37	3	MT	lead the United States
125.	8N1016	45.37	3	MT	Shandong Province
126.	8N106	45.37	3	MT	Russia
127.	8N924	45.43	3	MT	Guizhou Province
128.	8N025	45.50	3	MT	Hubei Province
129.	8N371	45.74	3	MT	Sichuan Province
130.	8N779	45.83	3	MT	Andalucía, Spain
131.	8N358	45.86	3	MT	Europe
132.	8N139	45.90	3	MT	Anhui Province
133.	8N127	46.03	3	MT	Jiangsu Province
134.	8N723	46.03	3	MT	Washington, United States

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135.	8N505	46.03	3	MT	Jiangsu
136.	8N217	46.30	3	MT	Jiangsu Province
137.	8N425	46.30	3	MT	Henan
138.	8N762	46.30	3	MT	Former, Soviet Union
139.	8N771	46.30	3	MT	Andalucía, Spain
140.	8N797	46.30	3	MT	Washington, United States
141.	8N848	46.30	3	MT	Washington, United States
142.	8N220	46.43	3	MT	Shandong Province
143.	8N735	46.43	3	MT	Andalucía, Spain
144.	8N208	46.46	3	MT	Hebei Province
145.	8N768	46.50	3	MT	Andalucía, Spain
146.	8N102	46.63	3	MT	Heilongjiang Province
147.	8N413	46.69	3	MT	Henan
148.	8N835B	46.69	3	MT	Washington, United States
149.	8N836	46.69	3	MT	Washington, United States
150.	8N1009	46.76	3	MT	Shandong Province
151.	8N829	46.91	3	MT	Washington, United States
152.	8N750	46.96	3	MT	California, United States
153.	8N1010	47.09	3	MT	Shandong Province
154.	8N519	47.09	3	MT	Egypt
155.	8N404	47.12	3	MT	India
156.	8N508	47.22	3	MT	Jiangsu
157.	8N749	47.22	3	MT	Turkey
158.	ZS-5	47.27	3	MT	Shandong Province
159.	8N239A	47.31	3	MT	Jiangsu Province
160.	8N168B	47.53	3	MT	Yunnan Province
161.	8N678	47.55	3	MT	Washington, United States
162.	8N259	47.57	3	MT	Jiangsu Province
163.	8N250	47.62	3	MT	Yunnan Province
164.	8N496	47.62	3	MT	Egypt
165.	8N317	47.75	3	MT	Ningxia
166.	8N493	47.75	3	MT	Washington, United States
167.	8N643	47.80	3	MT	Guizhou
168.	8N233A	47.84	3	MT	Hubei Province
169.	8N268	47.90	3	MT	Jiangsu Province
170.	8N790	47.94	3	MT	California United States
171.	8N189	48.13	3	MT	Gansu Province
172.	8N245	48.15	3	MT	Yunnan Province
173.	8N031	48.15	3	MT	Shanghai
174.	8N151	48.15	3	MT	Inner Mongolia
175.	8N578	48.15	3	MT	Kazakhstan
176.	8N752	48.15	3	MT	California, United States
177.	8N876	48.15	3	MT	Washington, United States
178.	8N888	48.15	3	MT	Washington, United States
179.	8N755	48.22	3	MT	Uzbekistan
180.	8N429	48.28	3	MT	Henan

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181.	8N178	48.32	3	MT	Hebei Province
182.	8N1044	48.47	3	MT	Shandong Province
183.	8N362	48.54	3	MT	Korea
184.	8N420	48.54	3	MT	Henan
185.	MS No1	48.64	3	MT	Beijing Shi, China
186.	8N409	48.68	3	MT	Henan
187.	8N626	48.68	3	MT	Guizhou
188.	8N736	48.68	3	MT	Razgrad, Bulgaria
189.	8N763	48.68	3	MT	Former Soviet Union
190.	ZS-8	48.74	3	MT	Shandong Province
191.	8N498	48.77	3	MT	Egypt
192.	DX CK	48.93	3	MT	Shandong Province
193.	8N274	48.94	3	MT	Jiangsu Province
194.	8N401	49.07	3	MT	Hebei Province
195.	8N759	49.07	3	MT	Nepal
196.	8N782	49.07	3	MT	California, United States
197.	8N784	49.07	3	MT	California, United States
198.	8N740	49.21	3	MT	California, United States
199.	8N239	49.34	3	MT	Jiangsu Province
200.	8N821	49.34	3	MT	lead the United States
201.	8N535	49.52	3	MT	Guizhou
202.	8N821	49.56	3	MT	Kazakhstan
203.	8N043	49.60	3	MT	Shandong Province
204.	8N132	49.60	3	MT	Yunnan Province
205.	8N796	49.60	3	MT	California, United States
206.	8N126	49.60	3	MT	Jiangsu Province
207.	8N785	49.60	3	MT	Burgas, Bulgaria
208.	8N511	49.69	3	MT	Egypt
209.	8N200B	50.00	3	MT	Hebei Province
210.	8N411	50.00	3	MT	Henan
211.	8N921	50.00	3	MT	Yunnan Province
212.	8N191	50.10	3	MT	Gansu Province
213.	8N610	50.13	3	MT	Shandong Province
214.	8N614	50.26	3	MT	Shandong Province
215.	8N066	50.33	3	MT	Shaanxi Province
216.	8N072	50.40	3	MT	Shaanxi Province
217.	8N186	50.53	3	MT	Hebei Province
218.	8N762	50.53	3	MT	California, United States
219.	8N947	50.62	3	MT	Yunnan Province
220.	8N612	50.66	3	MT	Shandong Province
221.	8N868	50.66	3	MT	Uzbekistan
222.	8N1015	50.79	3	MT	Shandong Province
223.	8N766	50.79	3	MT	Andalucía, Spain
224.	8N870	50.79	3	MT	Uzbekistan
225.	8N623	50.90	3	MT	Guizhou
226.	8N725	50.93	3	MT	Pakistan

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227.	8N531	51.11	3	MT	Guizhou
228.	8N893	51.16	3	MT	Lead the United States
229.	8N620	51.23	3	MT	Shandong Province
230.	8N298	51.32	3	MT	Yunnan Province
231.	8N644	51.32	3	MT	California, United States
232.	8N412	51.46	3	MT	Henan
233.	8N1008	51.72	3	MT	Shandong Province
234.	8N783	51.72	3	MT	Montana, Bulgaria
235.	8N035	51.85	3	MT	Shandong Province
236.	8N769	51.85	3	MT	Andalucía, Spain
237.	8N788	51.85	3	MT	Yambol, Bulgaria
238.	8N542	52.03	3	MT	Guizhou
239.	8N641	52.03	3	MT	Guizhou
240.	8N653	52.20	3	MT	North Macedonia
241.	8N275	52.38	3	MT	Beijing Shi, China
242.	8N732	52.38	3	MT	Beijing Shi, China
243.	JX	52.46	3	MT	Shandong Province
244.	8N439	52.47	3	MT	Henan
245.	8N361	52.73	3	MT	Europe
246.	8N377	52.78	3	MT	Sichuan Province
247.	8N885	52.78	3	MT	lead the United States
248.	8N030B	52.78	3	MT	Heilongjiang Province
249.	8N613	52.78	3	MT	Shandong Province
250.	8N238	52.84	3	MT	Jiangsu Province
251.	8N754A	52.91	3	MT	California, United States
252.	8N647	53.04	3	MT	Serbia
253.	8N867	53.09	3	MT	Uzbekistan
254.	8N236	53.15	3	MT	Hubei Province
255.	8N637	53.23	3	MT	Guizhou
256.	8N1020	53.44	3	MT	Shandong Province
257.	8N021	53.57	3	MT	Hubei Province
258.	8N440B	53.62	3	MT	Xinjiang
259.	8N509	53.70	3	MT	Jiangsu
260.	8N527	53.70	3	MT	Guizhou
261.	8N706	53.70	3	MT	Vermont, United States
262.	8N254B	53.78	3	MT	Yunnan Province
263.	8N403	53.84	3	MT	Hebei Province
264.	8N787	53.86	3	MT	Pleven, Bulgaria
265.	8N413-1	53.97	3	MT	Henan
266.	8N222	54.01	3	MT	Shandong Province
267.	8N219	54.26	3	MT	Shandong Province
268.	8N036	54.50	3	MT	Shandong Province
269.	8N130B	54.50	3	MT	Anhui Province
270.	8N263	54.50	3	MT	Jiangsu Province
271.	8N060	54.59	3	MT	Shaanxi Province
272.	8N027	54.63	3	MT	Jiangxi Province

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273.	8N044	54.63	3	MT	Shandong Province
274.	8N078B	54.63	3	MT	Thailand
275.	8N100	54.63	3	MT	Shanghai
276.	ZS-6	54.76	3	MT	Shandong Province
277.	8N506	54.98	3	MT	Jiangsu
278.	8N586	55.03	3	MT	Guizhou
279.	8N047	55.29	3	MT	Shandong Province
280.	8N066B	55.42	3	MT	Shaanxi Province
281.	8N024	55.42	3	MT	Hubei Province
282.	8N039	55.56	3	MT	Shandong Province
283.	8N1038	55.56	3	MT	Shandong Province
284.	8N892	55.56	3	MT	Lead the United States
285.	8N414	55.69	3	MT	Henan
286.	8N621	55.93	3	MT	Shandong Province
287.	8N536	55.94	3	MT	Guizhou
288.	8N541	56.44	3	MT	Guizhou
289.	8N424	56.48	3	MT	Henan
290.	8N428	56.48	3	MT	Henan
291.	8N590	56.48	3	MT	China
292.	8N502	56.72	3	MT	Hubei Province
293.	8N125	56.79	3	MT	Jiangsu Province
294.	8N231	56.79	3	MT	Hubei Province
295.	8N738	57.01	3	MT	Plovdiv, Bulgaria
296.	8N422	57.41	4	S	Henan
297.	8N503	57.78	4	S	Jiangsu
298.	8N406	58.02	4	S	Gansu Province
299.	8N312	58.20	4	S	Xinjiang
300.	ZS-9	58.60	4	S	Shandong Province
301.	8N556	58.64	4	S	Guizhou
302.	8N423	58.86	4	S	Henan
303.	8N587	58.91	4	S	Shandong Province
304.	8N002	59.16	4	S	Sichuan Province
305.	8N764	59.26	4	S	Yunnan Sheng
306.	8N512	59.47	4	S	Egypt
307.	8N273	59.57	4	S	Jiangsu Province
308.	8N421	59.66	4	S	Henan
309.	8N560	59.92	4	S	Guizhou
310.	8N930	60.05	4	S	USA
311.	8N514	60.05	4	S	Egypt
312.	JX-1	60.74	4	S	Shandong Province
313.	8N372	60.85	4	S	Sichuan Province
314.	8N076	60.98	4	S	Xinjiang
315.	8N566	61.11	4	S	Germany
316.	8N654	61.42	4	S	North Macedonia
317.	8N786	62.33	4	S	Burgas, Bulgaria
318.	WQS	63.16	4	S	Shandong Province

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319.	8N780	63.89	4	S	Syria
320.	8N629	63.89	4	S	Guizhou
321.	8N037	64.01	4	S	Shandong Province
322.	JX-4	64.20	4	S	Shandong Province
323.	8N002B	64.35	4	S	Sichuan Province
324.	8N410	64.51	4	S	Henan
325.	8N728	64.81	4	S	Former Soviet Union
326.	8N249	65.50	4	S	Yunnan Province
327.	8N975	65.74	4	S	Guizhou Province
328.	8N254A	65.78	4	S	Yunnan Province
329.	8N761	65.87	4	S	Former, Soviet Union
330.	8N218	66.14	4	S	Jiangsu Province
331.	ZSS	67.20	4	S	Shandong Province
332.	8N324	68.31	4	S	Yunnan Province
333.	8N013	68.58	4	S	Sichuan Province
334.	8N529	68.77	4	S	Guizhou
335.	8N427	69.75	4	S	Henan
336.	8N332	70.49	4	S	Yunnan Province
337.	8N561	70.55	4	S	Guizhou
338.	8N675	72.09	4	S	Poland
339.	8N526	72.41	4	S	Guizhou
340.	8N950	73.15	4	S	USA
341.	8N030A	73.41	4	S	Heilongjiang Province
342.	8N1040	73.81	4	S	Shandong Province
343.	8N545	75.80	4	S	Guizhou
344.	8N760	76.54	4	S	Greece
345.	8N559B	77.65	4	S	Guizhou
346.	8N1046	79.63	4	S	Shandong Province
347.	8N970	79.63	4	S	USA
348.	8N676	80.56	4	S	Syria
349.	8N1042	81.48	4	S	Shandong Province
350.	8N952	91.53	5	HS	USA
351.	8N649	95.37	5	HS	North Macedonia
352.	8N953	96.30	5	HS	USA
353.	8N972	96.30	5	HS	USA
354.	8N954	98.15	5	HS	USA

HT, highly tolerant; T, tolerant; S, sensitive; HS, highly sensitive

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