Effect of Sodium Chloride on the Panicle and Spikelet Morphogenesis in Rice

II. Developmental morphology of the panicle

Hengji CUI, Yoji TAKEOKA and Tomikichi WADA

(School of Agricultural Sciences, Nagoya University, Chikusa, Nagoya 464-01, Japan)

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Abstract : To provide new information on salt stress in rice plant based on developmental morphology, structural changes of panicle and spikelet were investigated. As the salt concentration increased, growth and development of the rachilet was progressively reduced. This resulted in a decrease in the number of primary and secondary rachilets and also decreased grain numbers in the panicle. Additionally, the percentage of fully ripened grains decreased as the salt concentration increased. Salt treatment caused morphological variations in rachilla, spikelet and leaf structures. Degenerated primary rachilets were decreased in size or abruptly reduced, although there was a wide range of variations in degree of the degeneration among different cultivars. These morphological changes in panicle and spikelet in rice plants under salt stress conditions are compared with those under other environmental stresses. There are many similarities in morphological variations under different stresses, which finally led to the inhibition of growth of rice plants. The common processes in morphogenesis of panicle and spikelet in response to these environments was discussed together with the previous knowledge.

Key words : Degenerated rachilet, Developmental morphology, Environmental stress, Glume, Panicle, Rice, Salinity, Spikelet.

イネの穂および小穂の形成に及ぼす塩化ナトリウムの影響 第2報 穂の発育形態について : 崔 亨吉・武岡洋治・和田富吉 (名古屋大学農学部)

要 旨 : イネの塩に対する反応を発育形態学的見地から研究するため、イネの穂と小穂の形成への塩化ナトリウム処理の影響を調査した。本報の実験材料と調製は前報と同様である。処理塩濃度の増加に伴い、枝梗と小穂の分化数が減少し、分化した枝梗と小穂でも、生長の減退したものが増加した。このため処理塩濃度が高くなるにつれて、一穂当たりの一次および二次枝梗および籾の数がいずれも減少した。また塩濃度の上昇により稔実歩合も低下した。塩処理により穂および小穂には、形態的異常が多様に発生した。例えば、一次枝梗の輪生と双生、穂最上位の一次枝梗の異常伸長、止葉の糸状化、無芒品種の芒生化、外穎と内穎の畸形化、小穂の半透明化および苞毛の脆弱化などが認められた。品種により一つの穂内の退化一次枝梗と正常な一次枝梗の大きさの差が大きい事例やほとんど差のないものが見られた。以上の結果をもとに、塩の影響と他の不良環境による影響とを比較したところ、不良環境要因が異なってもイネの穂および小穂の形態的変異には共通性のあることが認められた。これらの環境諸条件に反応する穂や小穂の形態形成過程について考察した。

キーワード : イネ, 穎, 塩類, 環境ストレス, 小穂, 退化枝梗, 発育形態, 穂。

It is commonly recognized that salt injuries in rice plants are caused by altered water absorption and by Cl or Na ion toxicity⁶⁾. Studies on the physiological mechanisms of salt tolerance are summarized by Shimose *et al.*¹²⁾ with reference to the histological basis, histologically specified structures and the morphological changes, and physiological aspects of the tolerance including osmotic pressure, nutrient deficiency and ion toxicity. Many biochemical studies have also been reported on plants in response to salt stress conditions. However, only a few studies have been undertaken concerning morphology of rice plants, especially with regard to panicles and spikelets under the influence of diverse

environmental stresses. We have previously shown common gross morphological changes in foliage and panicle of rice plants during panicle formation under salt stress²⁾. In this paper, we investigated the morphological changes of panicle and spikelet during the panicle forming stage under saline conditions. In the next paper, we will report on the developmental morphology of the internal features of the spikelet of rice plant.

Materials and Methods

As in the previous work²⁾, the same materials and methods were applied in this experiment. The experiment was carried out during 1992 and 1993, using three cultivars, Nippon-

Fig. 1. Effect of salt treatment on number of primary rachilets per panicle among the cultivars.

Fig. 2. Effect of salt treatment on number of secondary rachilets per panicle among the cultivars.

Fig. 3. Effect of salt treatment on number of grains per panicle among the cultivars.

Fig. 4. Effect of salt treatment on yield per panicle among the cultivars.

bare, Konosuke and/or Blue bonnet, grown in pots by means of sand culture with and without various concentrations of sodium chloride (NaCl). The salinity treatments were made during young panicle forming stage and from the beginning of panicle formation to its maturation. Samples were collected from both main stems and tillers of all plants cultured. Characteristics of panicle, such as primary and secondary rachilets, and the rachilets degenerated were examined, and panicle grain number and weight were estimated, and morphological characteristics of spikelets, in-

cluding the variation in size and shape of lemma and palea and the structure of degenerated spikelets were observed. In calculating degenerated and suppressed rachilet and spikelet structures, only Nipponbare was used.

Results

1. Panicle structures

There were some decreases in number of primary and secondary rachilets, and in grain number and weight per panicle as an increase in salt concentration treated (Figs. 1-4). However, in the cultivar Blue bonnet at a low

Fig. 5. Effect of salt treatment on percentage of ripening and sterility among the cultivars.

▨ Plump grain, ▩ Unripened grain and ▮ Empty grain.

salt concentration (0.1% NaCl) in comparison with the control ($P < 0.01$), the increase in number of primary rachilets, and also the increase in numbers and in weight of grain per panicle, was documented. This result is very interesting to compare with some stimulated growth in foliage regions²⁾.

It is clear from Figs. 1, 2, 3 and 4 that the wide variations of injury symptoms between two adjacent levels of salt concentration were varied. There were different salt effects from one concentration level to next level, depending on cultivar and salt concentration. For example, this aspect was evidently changed ($P < 0.01$) in the number of primary rachilets of cultivar Konosuke from 0.5% NaCl treatment to 0.7%, while the effect was only slight in the increase from 0.3% NaCl to 0.5% (Fig. 1). Moreover, the effect was significantly different ($P < 0.01$) in the number of secondary rachilets between the control and 0.1% NaCl level in cultivar Nipponbare, although the effect was only slight from 0.1% NaCl to the 0.5% range (Fig. 2). Focusing on the varietal difference, the number of primary rachilets was not so different between the control and 0.1% NaCl in Konosuke, and differed slightly in Nipponbare, but was very different ($P < 0.01$) in Blue bonnet (Fig. 1). It was found from Fig. 5 that the percentage of plump grains was decreased, while that of empty grains was increased with the increase in salt concentration. But change in the degree of unripened grains was different among the

cultivars. In Konosuke and Nipponbare, the percentages of unripened grains in 0.3% and 0.5% NaCl treatment were higher than those of the control and other concentrations treated. In Blue bonnet, it was much higher at 0.1% NaCl than the other concentrations. It is interesting to note that in Blue bonnet, the percentage of empty grains in 0.1% NaCl treatment was lower than that of control (Fig. 5).

For reference it should be noted there was an insignificant difference in the percentage of primary rachilets per panicle between 1992 and 1993, but it was obviously a significant difference ($P < 0.01$) in the secondary rachilets per panicle. In Nipponbare in the two years under 0.5% NaCl treatment, the mean values of primary rachilets per panicle were 6.10 and 6.00, respectively. But the value of secondary rachilets per panicle in the two years were 3.38 and 2.14. These values were especially higher with the increase under low salt concentration.

2. Degeneration in rachilets and spikelets

The degenerated secondary rachilets and spikelets were not counted separately, because it was not possible to count the degenerated secondary rachilets from samples collected at the panicle mature stage. The figures of the above two values together will be called 'primordia on primary rachilet', which did not include the degenerated spikelets on the reduced secondary rachilets. The percentage of the degenerated and suppressed secondary rachilets and spikelets was calculated and compared with those structures differentiated in the control plants. In this calculation, only Nipponbare was used. Fig. 6 shows both the percentage of degenerated and suppressed secondary structures on the primary rachilets and also indicates 'primordia on the primary rachilets'. The percentage of these structures mentioned above increased slightly with salinized solution. However, in primordia on the primary rachilets under low salt concentration, the difference between low salt treatment and control was absent or scarce.

From Fig. 7 c, d, it was found that degenerated primary rachilets were decreased in size and stopped the development in various stages. In Konosuke, these structures expressed as gradual along the rachilla within the

same panicle (Fig. 7d). But in Nipponbare, it was abruptly undeveloped (Fig. 7c). It was also clear from Fig. 7 that the degenerated rachilets and spikelets were located at the lower position of the panicle, and in the same rachilets, degenerated spikelets appeared at the basal region of the rachilet. On the morphogenesis under salt treatment, it was notable that panicle changed in structure as

verticillate formed primary rachilet, twined primary rachilet and abnormally developed primary rachilet at the top of panicle (Fig. 7 a, b). It was found in this investigation that in the cultivar Nipponbare under saline culture, three types of degenerated and reduced primary rachilet showed differentiation. The first type was both the secondary rachilet and spikelet primordia, differentiated on the re-

Fig. 6. The percentage of degeneration and suppressed differentiation of rachilets and spikelets as affected by the treatment of different concentration of salt in Nipponbare. pr : Primary rachilet. sr : Secondary rachilet. In this experiment 'sr' means 'primordium on primary rachilet'.

Fig. 7. Effect of salt on the rachilet morphogenesis of rice.

- a : Verticillate or twin primary rachilets.
- b : Abnormal development of primary rachilet at the top of panicle.
- c : Degenerated primary rachilet in Nipponbare.
- d : Degenerated primary rachilet in Konosuke.

Fig. 8. Type of primary rachilets degeneration resulted from salinity effect in Nipponbare.

- a : Both secondary rachilet and spikelets primordium.
- b : Only spikelets primordium.
- c : None of the above two.

Fig. 9. Malformation in glume under salt stress.

- a, c: Very poor developed lemma and palea laterally.
- b: Palea was poorly developed both laterally and longitudinally.
- d: Lemma and palea developed imbalance in the upper and lower part of the same glume.
- e: Normal grain.
- f: Very poor development of both lemma and palea longitudinally.
- g: Poor lateral and longitudinal development both of lemma and palea.
- h: Development of awn in awnless cultivar.

duced primary rachilet (Fig. 8a), the second type was only the spikelet primordia, differentiated on the degenerated primary rachilets (Fig. 8b), and in the third type, none of the above-mentioned structures had differentiated but only spikelet primordia had differentiated on the tip of the reduced primary rachilet (Fig. 8c).

3. Spikelet structures

Malformation in lemma and palea was observed as follows: Both lemma and palea developed poorly laterally (Fig. 9 a, c); only palea stopped the lateral and longitudinal growth (Fig. 9b); both lemma and palea developed imperfectly in the upper and lower part of the same spikelets (Fig. 9d); the awn developed abnormally in the awnless cultivar (Fig. 9h); both lemma and palea developed very poorly in the longitudinal direction (Fig. 9f) and both stopped the growth (Fig. 9g). It was interesting to note that bract hair and

flag-leaf formed irregular structures, such as the fragile, aggregated bract hairs of primary rachilets (Fig. 10a), translucent young spikelets (Fig. 10b) and filiform flag leaf (Fig. 10c).

Discussion

Results obtained in this experiment indicate that not only morphogenetic retardation but also organogenic malformation occurred in various portions of the panicle of rice cultured under high salinity conditions. This phenomenon means that under the influence of salinity stress, the rice plant responded in both differentiation and growth phases in young panicle, including spikelet morphogenesis. It was obvious that many types of the malformation in rachilet and spikelets shown in the result were one of the principal causes of the decrease in percentage of fully ripened grains in the panicle. The decrease in the number of primary and secondary rachilets on which spikelets were initiated was caused as a result of the reduction of development of rachilet.

Of the above mentioned conditions, it was interesting to note that the growth and development of the panicle were somewhat promoted in the cultivar Blue bonnet grown under low concentration of applied salt. Though the reason why such a developmental promotion was caused by relatively weak saline stress, even if only in this cultivar, is not clear at present, it is considered noteworthy as an aspect of the morphogenetic response of the rice plant to adverse environmental stress. The point is necessary to make clear in the near future investigation, as well as the difference among the rice cultivars used in this study focusing on the difference between Japonica, Javanica and Indica type of rice.

Almost of the malformed rachilets and spikelets observed in this study coincided with what was previously reported; verticillate or twin rachilets reported by Matsushima and Manaka⁷⁾ were caused by inorganic nutrition and environmental conditions during developmental stage from the first bract primordium to the end of reduction division, especially in the reduction division stage. According to their notice, this appearance was apparent under even good nutrition and environmental conditions. Many types of malformations of lemma and palea, as shown in this study, were

Fig. 10. Aspect of bract hair, glumous flower and flag leaf on salt treatment.
 a : The fragile, aggregated bract hairs of primary rachilet.
 b : Translucent young spikelet.
 c : Filiform flag leaf.

previously observed by Iwaki *et al.*⁴⁾, indicating deformation of flowers occurred under salt stress. These similar symptoms of morphological variations of lemma and palea may be indicated by depression of growth longitudinally and/or vertically, varying according to the degree of malformation. As indicated by Nakamoto *et al.*⁹⁾, there is a time lag in the growth period of glume between width and length, namely preceding in apical growth to form the length of glume, followed by marginal growth to form width. As a result, the shape of the lemma or palea, the degree of malformation, might be decided by the different ratio of these two dimensions of growth depression. Considering the suggestion of Nakamoto *et al.*⁹⁾ indicating that the degrees of growth in width and length of the rice glumes might be controlled by different genes, respectively, the different shape of the malformed lemma and palea, for example extremely slender ones not much shorter than the control, or where both length and width were extremely suppressed, may be supposed the result of a different process of response of the young spikelet under the interaction with these putative genes to saline stress. It is also supposed that the gene related width or length will interact more strongly at differing stages

in development of each young spikelet because the growth pattern of lemma was seen at different positions on the same panicle, as reported by Arai and Kono¹⁾. They studied the development of spikelet in relation to different positions on the panicle, and found that tendency and also that cultural conditions can improve the growth of the lemma on the lower position of the panicle. However, it is not clear how these two above-mentioned genes interact with one another, and how their gene expressions are controlled by environmental conditions. Concerning details of the possibility of this problem, it is necessary to undertake an intensive study for clarification.

Various malformations in glume have been widely reported with various kinds of environmental conditions, for example, water supply deficit¹¹⁾, malformation in glume observed in other environmental stress, such as water supply deficit¹¹⁾, transferring cultivation of paddy and upland field⁵⁾, drought^{8,17)}, low temperature^{15,16)} and gibberellin treatment¹⁰⁾. The fact that a similar type of malformation was caused in lemma by different types of environmental conditions, including exogenous gibberellin, indicates that there might be some common cause(s) in the occurrence of the malformations which would take part in the same

physiological mechanism of stress injuries induced by different environmental conditions. Even though the detail of the mechanism is not clear at present, the fact that each rice plant grown under different environmental conditions coincided with suppression of normal growth suggests the possibility of physiological activity caused under growth suppression, for example, synthesis and action of abscisic acid (ABA).

Considering the result of investigation by Iwaki *et al.*³⁾, which observed that Cl contents in leaves and stems were increased, and chlorophyll contents was by contrast decreased by salt treatment, the possibility is thought as that supply of assimilates produced by photosynthesis changed to insufficient, resulting in a deficit in nutrients for cell division and stem elongation in various organs in the panicle.

As revealed by Tabata *et al.*¹³⁾, reporting an awn development in awnless cultivar, and also a longer awn in an awn cultivar, respectively, by long day treatment, they point out the awn is also easy to develop by environmental condition, even if the development of this organ is hereditary character under genetic control. Takahashi *et al.*¹⁴⁾ is suggestive on this point, remarking that possessing an awn or not is a hereditary characteristic but frequency of appearance and length is affected by environmental condition.

Many investigations have been undertaken determining the influence of environmental conditions as mentioned above on the inner structure of the rice spikelets. We will also report successively on the variations in morphogenetic features of the inner spikelet organs, especially stamens and pistil focusing on their sex expression as affected by the salt stress treatment.

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