Different Responses of Soybean Plants to an Excess of Water with Special Reference to Anatomical Observations.

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The writer reported previously that TSURUMAME, Glycine usuriensis is much more tolerant against excess-moisture injury than Kurogoki, G. hispida (1), and, that a conspicuous aerenchyma, which is originated from the secondary meristem homologous with phellogen, has spread over the periderms of the stem, hypocotyl, roots, and nodules of TSURUMAME growing wild under swamp conditions (2). He further reported on an aerenchyma derived from the fundamental meristem in the rice plants (3). The present paper deals with the effect of soil moisture on the development of the aerenchyma and adventitious roots with special reference to anatomical observations, and its relation to tolerance against excess-moisture injury in soybean plants. Before proceeding further the writer wishes to express his hearty thanks to Drs. S. Ueda and M. Yamazaki for their giving him many encouraging suggestions.

Materials and Methods

In 1952, the experiment was conducted in a greenhouse, by using SHIRODAIKEU, G. hispida, and TSURUMAME. Preparations for sowing given were almost similar to those in the previous experiment (1), excepting that 110 g of dried barnyard manure was applied to each pot in order to keep the soil much reduced when flooded. The water content of the soil, which was controlled by weighing method, was brought to 50 per cent of the water capacity for dry plots and 90 per cent for moist ones that were switched to flooded plots on and after July 17. Besides, another two set by exposing the plants alternately to dry and to flooded conditions at intervals of one or two months, being designated as the "flooded-dry-flooded" and "dry-flooded-dry" plots respectively. In case of requiring the flooded condition the soil was immediately covered with water about 2 cm deep above the soil surface. On the other hand, the following way was taken to change the soil moisture from the flooded condition to the dry one: After the soil was drained off on the appointed day, no watering was done for several days until its water content attained to 50 per cent of the water capacity, then it was watered again twice a day to the definite level of the water content. Each plot consisted of five pots. To each of them six seeds were sown July 1. After germination occurred they were thinned to the two and three most vigorous plants per pot; two for SHIRODAIKEU and three for TSURUMAME. The measurements of the temperatres and redox-potentials of the soil were taken. At the end of the period of each treatment one pot was assigned for collecting test plants necessary for anatomical observations. The whole appearance and cross-sectional views of the hypocotyl, primary roots, and adventitious ones were diagrammatically drawn by the aid of free-hand sections, using fresh material (4, 23, 28). In the present paper, however, the readers are referred to a restricted number of illustrations owing to the necessity of curtailment of the paper. As the writer has been compelled to despise the investigation of yield, putting emphasis on anatomical observations, the results on yield shown in Table I may not provide us with reliable data to denote different degrees of adaptability to the various treatments between the two species.

* Received April 2, 1953.
Nevertheless, he has thought it worth while to collect numerical data which should serve to indicating the relative tolerance of these plants against excess-moisture injury.

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<th>Table 1. Results of the harvest (air-dried material in gramme)</th>
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The following abbreviations are used on all illustrations: Ae, aerenchyma; ar, adventitious root; ca, cambium; cae, collapsed aerenchyma; cco, collapsed cortex; cr, crack; en, endodermis; ep, epidermis; le, lenticle; me, meristem; mr, medullary ray; mx, metabolylem; pe, pericycle; pi, pith; pp, protophloem; rn, root nodule; sc, sclerenchyma; si, sieve portion; sm, secondary meristem; ss, soil surface; v, vessel; vp, ventilating pit; wo, wood portion; wi, water line.

The shaded portions in the figures are referred to the tissues which were perished, and the dotted portions to the ones having intracellular spaces filled with air.

Experimental results

(1) Flooded-dry-flooded plot  SHIRODAIZU—Soon after the soil had been flooded July 17, conspicuous symptoms of injury became visible on the plants. The lower leaves were yellow and some were abscising, and the leaf cushion of the middle leaflet showed hypostomatic curvature. These symptoms began with the lower leaves and progressed upwards along the stem. The plants assigned for anatomical observations were washed out of the pot July 28. As a effect of excessive water supply attended by diminution of air, the primary roots were then found to have decayed or almost been killed. They were tinged with blue black, indicating deposition of Fe-S originated in the combination of iron previously absorbed into the roots with H₂S which was generated in the soil after flooding (Fig. 1). Hence, the growth of the top portion was much retarded, keeping only one or two leaves alive at the uppermost position. The epidermis of the hypocotyl near the water surface was dotted with aerenchyma just similar to lenticle in its appearance (Fig. 2), through which a few adventitious roots weak in vigor projected into water. Furthermore, lumps of ventilating pits were visible on the stem. Within about two weeks after the soil was drained off Aug. 1 and its water content was brought to 50 per cent of the water capacity, all of the leaves were dried up, resulting in a complete destruction of the whole plant (Plate 1).

TSURUMAME—The plants which had been growing vigorously till July 17 showed just similar symptoms of injury when they were flooded, as these already remarked in SHIRODAIZU. The aerenchyma which was originated from the secondary meristem began to extend over the periderm of the hypocotyl above and under the water line. The presence of the steadily expanding aerenchyma caused the cortex and epidermis to bulge outwards, and finally to burst over the fully developed aerenchyma (Fig. 4). A few days later, adventitious roots projected into water one after another. On the other hand, the primary roots had mostly been killed when the plants were taken out of the pot July 28, although some of them were still alive developing the aerenchyma on the periphery of their old portions just under the soil surface (Fig. 3). Naturally, the adventitious roots enabled the plant to resume normal growth superseding the dying primary roots. They did
not run deeply into the soil but supported themselves in the water or in a shallow layer of the surface soil.

After Aug. 1, when the soil was drained off in order to keep the moisture as low as 50 per cent water capacity, the plants recovered themselves with a sharp contrast to the complete destruction of the whole plant in *Shirodaiyu* grown under the same conditions. The aerenchyma developed on the aerial part collapsed gradually with the change of the secondary meristem into phellogen, while that developed underground was not always perished being protected from desiccation. At the end of the period of this treatment, the adventitious roots which had been floating in the water during flooding treatment were found to have grown deeply in the soil and established a considerably favorable root system, as if they were the primary roots (Fig. 5). That is the reason why the plant has been able to continue its normal growth instead of being destroyed by the radical change of moisture content of the soil (Plate 2).

The plants were immersed again Sept. 1. They showed modifications in response to flooding: Phellogen was replaced by a meristem that gave rise to the aerenchyma and, a few days later, secondary adventitious roots grown out newly. The first adventitious roots which had been growing deeply in the soil before it was reflushed were almost killed (Fig. 6). Accordingly, the active root system at the harvest time consisted mostly of the secondary adventitious roots thus formed (Plate 3).

(II) **Dry-flooded-dry plot** *Shirodaiyu*—The plants showed normal growth while they were growing under the dry condition of the soil. No hypertrophy could be seen on any parts of the stem and roots. The primary roots extended deeply into the soil, and their old portions became gradually suberized. Immediately after Aug. 1, when the soil was covered by water, however, the plants began to reveal the injurious symptoms of flooding. The primary roots were
so much injured by the decline of the redox-potential of the soil that they became completely perished, and moreover the adventitious roots were not produced so many as in 
Tsurumame, consequently resulting in a poor growth of the top portion. As shown in Fig. 7, it seems that the bud initial has been converted into an adventitious root. But it must be comprehended that an extra meristem has been formed in the stem above the axillary bud, and this meristem has grown into a root, as Howard (18) pointed out in Brassica. The plants failed in continuous growth soon after Sept. 1, when they were exposed again to the dry condition of the soil. It may be correct to consider that a poor development of the adventitious roots in the preceding period should be responsible for this failure.

Tsurumame——The plants grew as favorably as those of shibodaizu, in July. When immersed Aug. 1, however, the typical symptoms of injury were soon recognized. Although this treatment gave a destructive effect on the primary roots, it promoted the production of the aerenchyma and adventitious roots in such a way as that already described. The effects of the flooding treatment in August on the plant growth, especially on the seed production, were more detrimental than those in July. It may in part be attributable to the prolonged duration of the flooding treatment in August as compared with that in July. We must further consider the fact that the floral organs of the two species were initiated in August, which would have been a critical period from the view point of plant physiology (12).

After Sept. 1, the plants were continuously subjected to the dry condition of the soil until they reached full maturity. As time went on, the aerenchyma became perished on account of interruption of water supply by a cork layer which appeared beneath the aerenchyma (Fig. 8). It was also revealed that a large number of adventitious roots had extended favorably into the soil when the plants were taken out of the pot Nov. 10 (Plate 3). It is in virtue of these reformation that the plants have been able to maintain a complete continuation of growth until they reach full maturity.

(III) Continuously flooded plot Shibodaizu——The primary roots were rendered incapable of carrying on their own functions when covered by water, being afflicted with a severe reduction of the soil. The hypocotyl and the lower part of the stem were dotted with the lenticel-like aerenchyma and ventilating pits. Inasmuch adventitious roots which to supersede the affected
primary ones were neither produced abundantly nor able to grow fairy. the plants were eventually perished in September in spite of their having persevered in adverse circumstances for a fairly long time.

Tsurumame —— The destruction of the primary roots owing to poor oxygen supply was accompanied with the typical symptoms of flooding injury. As soon as a conspicuous aerenchyma and numerous adventitious roots were produced, however, the plants began to resume normal growth. They could grow up to maturity without interruption. These favorable recuperation processes made it possible for the plants to yield best, as shown in table 1. The adventitious roots also gave rise to the aerenchyma on their periderms as they grow older, but they did not grow deeply in the soil (Plate 3). The roots grown in the soil with a moderate moisture content are able to utilize much more nutrients than the floating ones by absorbing mineral salts that are to be solved by acid substances secreted from the roots and nutrients derived from organic materials due to biochemical oxidation, in addition to dissolved nutrients in water, that is, the very source of the nutrients available for the floating roots like the adventitious roots under consideration. Thus, not only the depression of physiological functions in the plant-body caused by reduced conditions of the soil, but also the above-mentioned inferiority in the nutrient absorption, may be considered as the factors concerned in a decreased yield of the flooded plants as compared with the ones grown in the soil with moderate moisture (1). Not infrequently, activity of the meristematic layer that produces the aerenchyma degenerates in a shorter length of time as the plant grows older and the temperature falls with approaching maturity. In this case, it is occasionally renewed by a freshly formed layer of the same kind, as illustrated in Fig. 8. The innermost layer only remains active as a meristem.

(IV) Continuously dry plot SHIBODAIU and Tsurumame —— The water content of the soil in this plot was always kept at the 50 per cent level which would be enough to ensure a healthy growth (Plates 1, 2). A relatively low moisture content of the soil stimulated the root development, resulting in a greatly increased absorbing surface (Plate 3). As the plant grow older, the old portions of its roots were covered by cork which was invariably derived from the cell division in the pericycle (Fig. 9). It must be pointed out, however, that the cork as well as the aerenchyma on the stem portion in SHIBODAIU was raised from an optional layer of the cortical cells by irregular division, whereas in Tsurumame they were produced from the pericycle or the endodermis and pericycle combined, which were closely adjoining to the sclerenchyma or protophloem, being provided with a continuous meristem. It was difficult
to make generalization concerning to the tissues from which the adventitious roots originate.

Fig. 9.

Discussion

An adequate supply of oxygen to the roots is essential for growth of plants. Probably, there are various ways by which the plant roots are supplied with oxygen, the distinction being a criterion of ecological importance which separates the plant groups. Especially, we must pay much attention to the degree with which oxygen comes down from the top to the roots. As Karsten has remarked, the breathing roots of *Somerutia* may be said as if a large lenticel had spread over the whole surface of the organ (16). It is probable that the aerenchyma herein reported carries on gaseous interchange among the parts afflicted with reduced oxygen supply. Some investigations on this problem are now in progress.

Sugawara (36) assumes that the roots of soybean plants are so intense in the power of reducing nitrate that they require a very large quantity of oxygen for respiration. On the other hand, Ueda (39) reported that soybeans grew best in the soil whose water content was always kept at 90 percent of the water capacity throughout the whole stage of growth. It is said that soybean plants are not only highly resistant against drought but also tolerant to excessive moisture in soil (9). Neither large air spaces nor aerenchyma could be found in the cortex of the normal stem (Fig. 10). So it may be affirmed that they are essentially less tolerant against excessive moisture in soil because of lower ability for oxygen transportation from the top down to the roots. As already stated, however, Tsuruma is able to adapt itself to a wet place by generating a conspicuous aerenchyma and further developing adventitious roots, if the primary ones were going to be damaged by a severe reducing condition of the soil. From the anatomical point of view, it may be correct to assume that the soybean plants, including *G. hispida* and *G. usuriensis*, have varying abilities to accommodate themselves to habitats different in the soil moisture. They develop the cork layer, in case the soil is kept in dry conditions and, on the other hand, they give rise to the aerenchyma, in case the soil is under reducing condition, on account of excessive moisture. The two extreme conditions are connected by various intermediate modes of structural adaptation, that is, *G. hispida* may vary widely in the ability of developing aerenchyma in accordance with the varying degree of tolerance against excessive moisture. Kurosendoku, Yahagidaizu, and some other varieties are, for instance, able to produce the spread-over aerenchyma just similar to that of *G. usuriensis* when soil is saturated by continued rainfall. The aerenchyma formed on the stem and roots does not always concomitantly induce the production of adventitious roots. There is no necessity for producing them because the primary roots are still active in their functions.

Flooding probably stops the downward translocation of carbohydrates, nitorgenous compounds and
auxin, and causes their accumulation at the water line, favoring the induction of root primordia, development of aerenchyma, and, in consequence, formation of adventitious roots (7, 10, 22). When the aerenchyma is not provided with a continuous meristem, as in shibodaihu, few root primordia are formed and they hardly grow into adventitious roots perhaps owing to poor oxygen supply.

Recent activity in the study on plant hormones has revealed the reaction of plant tissues to the substances under various environmental conditions (8, 13, 15, 17, 21, 26, 27, 31, 32, 41, 42). Referring to them with other numerous reports concerning the formative effects of synthesized bhemicals, the writer considers that the anatomical features adapting to an excess of water, so far observed in the soybean plants, may certainly be attributable to the effect of growth promoting substances.

Adventitious roots of soybean plants which take the place of the dying primary roots grow only in the water or in the surface soil, showing no sign of extending into deep strata of soil under reducing condition where the \( E_{H_2} \) value is lower than 0.2 volt. (Figs. 6, 7, and Plate 2). The tips of the primary roots growing in a well aerated soil do not abound in air spaces in their cortex (Fig. 11), and their old portions are covered by cork (Fig. 12). On the contrary, the tips of the adventitious roots growing in the soil covered with water are provided with abundant schizogenic intercellular spaces (Fig. 13), and in their old portions the central cylinder is surrounded by aerenchyma which originated from the pericycle (Fig. 14). It has generally been assumed that the air spaces result in a more adequate supply of oxygen for the cells of roots (6, 22, 28, 29, 43).

Harberlandt (16) described that it would not be correct to assume that there is always a close correspondence between the average intensity of respiration and the degree of development of the ventilating spaces.

Besides, Kramer (22) states, “it is quite possible that an important reason for survival of roots developed under water is that they are physiologically different from those produced in well aerated media and perhaps because of differences in their respiratory enzyme system they can actually function with a lower supply of oxygen.” Tomi (38) reported that a high feeding variety of soybeans is more intensive in root respiration than a low feeding variety, under upland conditions. From the physiological standpoint, investigations will be carried out in the future.

**Summary**

(1). Tsurumame, the wild soybean develops a conspicuous aerenchyma on the stem portion, through which many adventitious roots grow out superseding the dying primary roots, when
flooded. The aerenchyma originates from a secondary meristem homologous with phellogen.

(2). **Shiroyaize**, the cultivated soybean, is inactive to generate the aerenchyma provided with a continuous meristem. It produces a lenticel-like aerenchyma or ventilating pits. Both of them are produced from an optional layer of the cortical cells by irregular division. The aerenchyma may act as a breathing root or the lenticel in its function.

(3). The aerenchyma once formed during an over-moist period is readily replaced by the cork when the soil becomes dry, and reciprocally, the cork tissue formed during the dry period is broken by the aerenchyma when the soil is flooded.

(4). Soybean plants, in general, have on aerenchyma in the cortex of the stem and roots under upland conditions, giving an evidence for not vital transportation of oxygen from the top down to the roots.

(5). Adventitious roots growing in water are characterized by abundance of air spaces in the cortex of their tips and well-developed aerenchyma derived from the pericycle in their old portions. Even these roots equipped with ventilating spaces are unable to survive in the soil of reducing character where the roots of rice plants may surely be able to grow.

(6). The activity of the secondary meristem to produce aerenchyma declines in accordance with the age of the plant and with the circumstances under which the plant is placed. Plant hormones may be influential upon the generation of this activity.

(7). The varietal difference of this plant in tolerance against excessive moisture may in part
大豆の過湿に対する変態, 特にその解剖学的差異について *

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摘要

（1） 過湿性の大なる大豆は浸水状態では第一次根の黒死に伴い, 茎根部に顕著な通気組織を形成し, 之を貫通して多数の不定根が生ずる。通気組織は根皮形成層と同質なる第二次形成層から発生する。

（2） 過湿性の小なる白大豆では皮目の如き通気組織を局部的に形成的に過湿し, 且つ, 不定根の発生も少、第二次形成層の分化は不明瞭である。大豆に見られる通気組織は一種の呼吸根根の皮目の如き作用を営む。

（3） 大豆茎部の厚皮中には通気組織と認めべき顕著な細胞間隔は認められず, 従って大豆は木質的に茎葉から根部に同様の薄葉状の根系は観察され易い形態を具えていないと考えられる。

（4） 根皮形成層は環境に応じ, 乾燥時には根皮を生じ, 湿潤時には通気組織を形成する。斯る第二次形成層の活動はホルモンの影響によるものと考える。

（5） 水中に生育する不定根の先端部に生じた厚皮は分離細胞間隔に富み, 且つその古い部分では内類の分裂により通気組織が形成されている。之等の不定根に醜葉不足の土壌中に深く進入することは不可能である。

（6） 大豆の過湿性の大小は通気組織及び不定根の発生の難易により或る程度決定されるものと考える。

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