Jpn. J. Crop Sci. 65(2) : 309—314 (1996)

Changes with Aging in the Activities of Succinic Dehydrogenase and Peroxidase in Rice Seminal Root System*

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Received August 18, 1995

Abstract : Aging pattern of rice seminal root system grown under submerged soil conditions was evaluated by the measurement of succinic dehydrogenase (SDH) and peroxidase (POD) activities with special reference to the difference between the basal 11 cm-portion of the seminal root axis (SRA) and the lateral roots (LR) initiated from this portion. SDH activity in both SRA and LR showed high levels just after their initiation, and subsequently these activities decreased sharply with aging. The activity in LR always exceeded that in SRA. The percentage of the LR that positively reacted to the reduction assay of triphenyltetrazolium chloride was 100% in the first 4 days after initiation, followed by a drastic decrease ranging from 14 to 24%. Even at heading stage, however, about 8% of the LR still showed a positive reaction. In contrast, POD activity in both SRA and LR increased with aging. The activity in LR, again, always exceeded that in SRA, and attained the highest level which was 3.6 times higher than that in SRA at heading. This elevation of POD activity with aging was discussed in terms of induced protective reaction which may delay senescence. These results indicated that the senescence of SRA precedes that of LR, and LR plays a major role in the physiological activity of the seminal root system. In addition, the rice seminal root system was found to be alive up to the heading.
Key words : Aging, Lateral roots, *Oryza sativa* L., Peroxidase, Root, Seminal root, Senescence, Succinic dehydrogenase.

水稲種子根系のエイジングに伴うコハク酸脱水素酵素およびパーオキシダーゼ活性の変化 : 郭　康洙・飯嶋盛雄・山内　章・河野恭廣（名古屋大学農学部）

要　旨 : 湛水土壌条件下で成育させた水稲種子根系のエイジングのパターンを明らかにするために，基部から 11 cm部分の主根軸とそれから発根した側根を対象に，両者のコハク酸脱水素酵素（SDH）およびパーオキシダーゼ(POD) 活性の変化を経時的に調べた．主根軸と側根におけるSDH活性は，発根直後は高い活性を示したが，その後はエイジが進むにつれて急速に活性が低下した．また，側根の方が常に主根軸より高い活性を維持した．Triphenyltetrazolium chloride 還元反応に対して陽性反応を示した側根の割合は，発根後4日までは100%であった．その後は14から24%に低下したが，出穂期においても依然として約8%の側根が陽性反応を示した．一方，主根軸と側根における POD 活性は，エイジの進行とともに増加した．SDH 活性と同様に側根が常に主根軸の活性を上回り，出穂期においては最高値に達し，約 3.6 倍の活性を示した．このようなエイジングに伴う POD 活性の上昇について，とくに老化遅延との関連で考察した．これらの結果は，側根より主根軸の老化が先行することと，側根が種子根系の生理的活性において重要な役割を果していることを示唆した．さらに，水稲の種子根系は少なくとも出穂期まで生存していることが明らかとなった．
キーワード : イネ，エイジング，コハク酸脱水素酵素，種子根，側根，根，パーオキシダーゼ，老化．

Succinic dehydrogenase (SDH) is an enzyme in the tricarboxylic acid cycle and involved in respiratory activity. SDH activity has often been used to determine the viability of senescent tissues as well as damaged plant tissues induced by environmental stresses[14,15]. When SDH activity is determined as an index of the viability of crop roots, triphenyltetrazolium chloride (TTC) reduction assay has often been employed owing to its close relationship to SDH activity. Using TTC reduction assay, Jacques and Schwass[7], and Joslin and Henderson[9] discriminated living roots from dead ones. Knievel[11] also reported that the amount of TTC reduction per unit dry weight of living corn root decreased as the root age progressed.

On the other hand, peroxidase (POD) is one of the most abundant enzymes in plants, and has been shown to be involved in the senescence of plants via participation in the biosyntheses of ethylene and lignin, and oxidation of indole-3-acetic acid[4,5]. In fact, the

* An outline of this paper was presented at the 199th meeting of the Crop Science Society of Japan held in April, 1995.

activity of POD generally increases as plant cells grow and mature[3,17]. Due to characteristics mentioned above, POD activity has been referred to as one of the most reliable indicators of plant senescence.

Yoshida and Takahashi[20,21] demonstrated that in a 9 to 10 days-old rice seminal root system, SDH activity and the concentration of functional substances such as protein, phosphorus and sugar along the root axis increased acropetally. In contrast, POD activity and the concentration of elements that constitute the cell wall, such as cellulose and hemicellulose along the root axis, increased basipetally. Based on these facts, they proposed that the activities of SDH and POD can be used as indices to represent the aging of roots ; the higher SDH activity indicates that the root age is younger, while the higher POD activity much older.

These results strongly suggest that both enzyme activities in a root system are age-dependent. Therefore, it is of interest to estimate the activities of SDH and POD in a root system in relation to aging.

Although Yoshida and Takahashi[21] compared the activities in different portions along the seminal root axis, no attention was paid to the difference between the root axis and its concomitant lateral roots. In cereal crops, lateral roots account for most of the length and surface area of the entire root system and play an important role as an absorbing organ for water and minerals[10,16]. Our previous study[13] indicated that in the rice seminal root system, metabolic activity in terms of carbon and nitrogen concentrations in the lateral roots was much higher than that in the root axis, and the senescence of the root axis preceded that of the lateral roots. Therefore, it is reasonable to assume that the activities of SDH and POD in lateral roots would also be different from those in root axes.

In this study, therefore, we determined the activities of SDH and POD as marker enzymes to estimate aging patterns in the seminal root axis and its concomitant lateral roots and also to evaluate the longevity of the seminal root system of rice plant grown under submerged soil conditions up to heading stage.

Materials and Methods

Seeds of rice plants (*Oryza sativa* L. cv. Aichiasahi) were sown in root boxes filled with loamy sand soil on June 7, 1994, and the plants were grown under submerged conditions up to heading stage (September 2) in a vinyl-house. The root systems were sampled every day during the first 7 days after sowing (DAS), and thereafter at 10, 12, 14, 21, 28, 35 DAS and the heading stage. The size of root boxes was 25 cm in length × 2 cm in width × 40 cm in depth for the plants sampled until 35 DAS, and 45 cm in length × 3 cm in width × 50 cm in depth for those sampled at heading stage. For the plants sampled until 35 DAS, three to 10 plants were grown in each root box, depending on the growth period and no fertilizer was applied. For the plants sampled at heading stage, however, only one plant was grown in each root box and 2 g of compound fertilizer (N, P_2O_5, K_2O ; 12%, 16%, 14% in weight) were applied to each root box at 35 DAS. The root systems were sampled by using the root box pin-board method[12] that allows the sampling with a minimum loss of roots. At each sampling, the root systems from 10 to 15 replicate root boxes were sampled depending on the growth period. At heading stage, only eight replicates were used.

In this study, we dealt only with the seminal root system for the analysis. It was, however, often very difficult to identify the seminal root axis grown for more than one month because of the great volume of the root system. Therefore, to facilitate the identification of seminal root axis among the great number of nodal root axes, pre-germinated seminal root axis was led to a hole made on a small piece of polyethylene film upon sowing.

The root portions that were subjected to the enzyme assay at different sampling times are summarized in Fig. 1. On 1 and 2 DAS, the sampled seminal root was divided into 1 cm-root tip and the rest of the roots. Thereafter from 3 DAS, since the lateral roots started to emerge on the seminal root axis, they were cut apart from the root axis using fine forceps. That is, these seminal root systems were divided into three root portions ; seminal root axis, lateral roots and 1 cm-seminal root tip. From Day 7, the collected root portions were the basal 11 cm part of seminal root axis (SRA), which was equivalent to the length elongated in the first 6 days, their lateral roots (LR) and 1 cm-seminal root tip, although the

root tips were excluded after 21 DAS. All procedures, such as handling root specimens, were done under continuous chilled conditions with ice. All of the samples were above 100 mg in fresh weight.

We followed the assay procedures of SDH and POD activities proposed by Yoshida and Takahashi[19,21]. All of the extraction procedures of POD were conducted under chilled conditions. Formazane and tetraguaiacol which are produced in the assay for SDH and POD activities, respectively, were determined spectrophotometrically with the absorbance at 470 nm (UV-160, Shimadzu Co. Ltd.). SDH activity was expressed in micromoles of formazane which is produced through the reduction of TTC by SDH. At each sampling, the intact seminal root systems from five plants were tested histochemically for the reaction to TTC reduction assay[8] and the number of LR which positively reacted to TTC were counted under a stereo microscope. However, at the heading stage only one seminal root system could be used to the reaction test. For POD assay, the activity was expressed in guaiacol units (G.U.). One G.U. is equivalent to the amount of the enzyme that produced 1 mg of tetraguaiacol.

In addition, the seminal root systems from five to ten plans were fixed with FAA at each sampling, and the length and number of LR which were emerged on the SRA were determined.

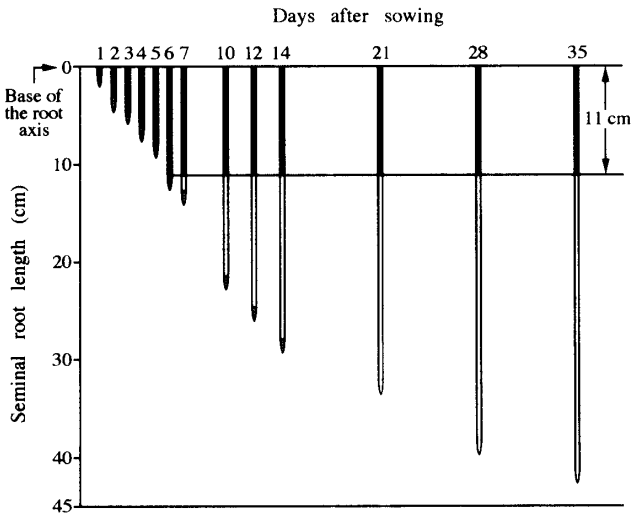

Fig. 1. Schematic diagram of the seminal root elongation pattern with aging. For the enzyme assay, the seminal root axis portion which elongated in the first 6 days after sowing (DAS), i.e. 11 cm from the root base was used. In the root portion, seminal root axis and lateral roots grown on this axis portion was used. One cm-seminal root tip was also used till 14 DAS. In the case of seminal roots before 6 DAS, however, 1 cm-root tip and the rest of seminal root portion were used for the assay.

Results

SDH activities of both SRA and LR were very high levels at the beginning (Fig. 2). Thereafter, the activities decreased drastically and maintained low levels. The activity in LR always exceeded that in SRA until 35 DAS. One cm-seminal root tip showed high SDH activity for the first 7 days ranging between 3.4 and 4.1 μM formazane which were much higher as compared with those of SRA and LR. Thereafter, the activity also decreased rapidly to 2.3 μM formazane at 14 DAS.

In TTC reaction assay which is closely related to SDH activity, all of the LR showed positive reaction till 4 days after initiation (Fig. 3). Thereafter, the percentage of positively reacted LR decreased sharply as the pattern of SDH activity shown in Fig. 2, and only 14 to 24% of the LR showed the positive reaction from 21 to 35 DAS. Those positively reacted

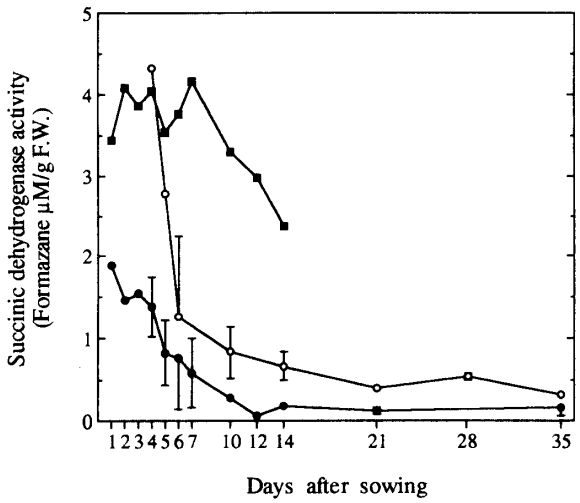

Fig. 2. Changes with aging in the succinic dehydrogenase activities of SRA (●—●), LR (○—○) and 1 cm-seminal root tip (■—■). The activity was expressed in micromoles of formazane per gram fresh weight of roots, which was produced by the reduction of TTC by SDH. Each value represents the mean of three replicate measurements ± standard deviation, however the value for root tip represents only one.

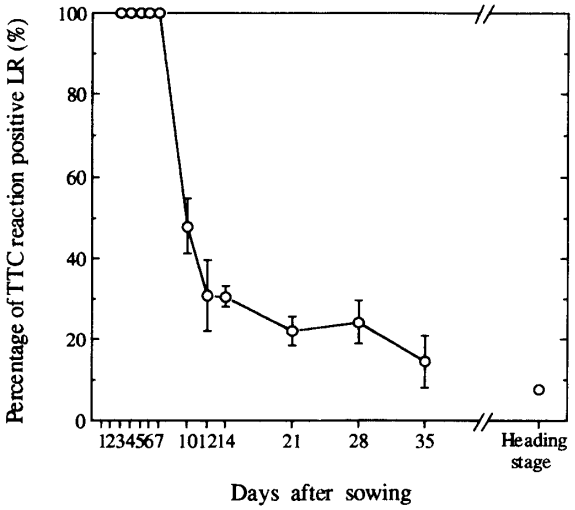

Fig. 3. Changes with aging in the percentage of TTC reaction positive lateral roots (LR) which initiated on the basal 11 cm portion of the seminal root axis. LR that showed positive reaction only partially along the root were also counted as positive LR. Values from 3 to 35 days after sowing represent the mean value of five replicate measurements ± standard deviation. The value at heading stage is from one plant, so that the error bar is not presented.

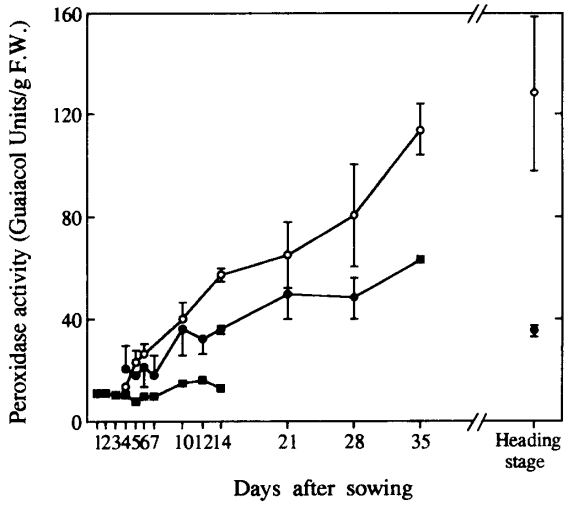

Fig. 4. Changes with aging in the peroxidase activities of SRA (●—●), LR (○—○) and 1 cm-seminal root tip (■—■). The activity was expressed in guaiacol units (G.U.) per gram fresh weight of roots. One G.U. is equivalent to the amount of the enzyme necessary for producing 1 mg of tetraguaiacol. Each value represents the mean of three replicate measurements ± standard deviation, however the value for root tip represents only one.

LR were stained predominantly in their root tips. Even at heading stage, however, about 8% of the 1st order LR showed positive reaction partially along the root axis (Fig. 3).

In contrast to SDH activity, POD activities of both SRA and LR showed increasing patterns with aging (Fig. 4). The activity in LR again always exceeded that in SRA after 4 DAS till the heading stage. As a result, the activity in LR at the heading stage was 3.6 times (128 G.U.) that in SRA (35 G.U.). In 1 cm-seminal root tip, the activity did not show considerable changes during the measurement period till 14 DAS ranging between 10 and 20 G.U., which were much lower as compared with those of SRA and LR.

The growth in terms of elongation and initiation of 1st order LR almost ceased after 12 DAS, which was then followed by the development of the 2nd order LR initiated on the 1st order LR (Fig. 5). Synchronized with the

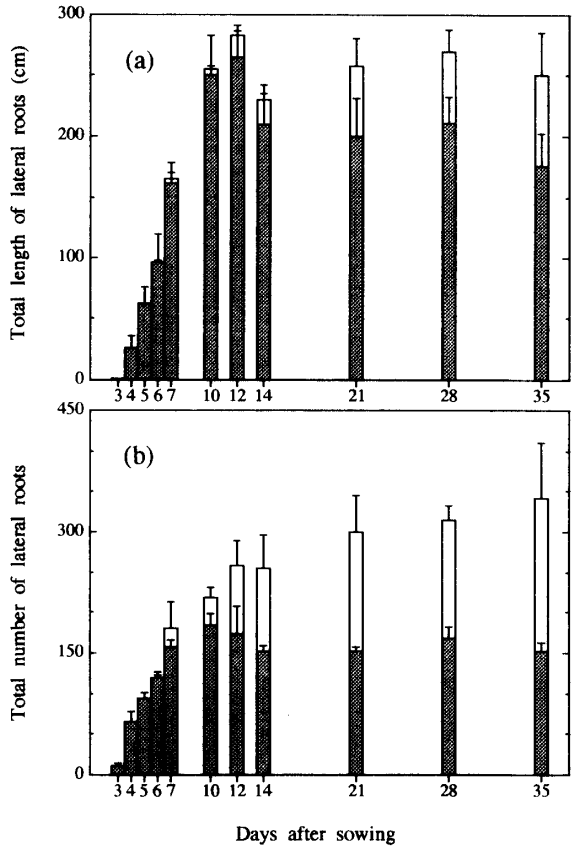

Fig. 5. Changes in the length (a) and number (b) of 1st (▨) and 2nd (□) order lateral roots which initiated on the basal 11 cm portion of single seminal root axis. Each value is the mean of five to ten replicate measurements. Bars indicate standard deviation.

emergence and subsequent development of the 2nd order LR, the activity of POD in LR increased continuously, although data on the growth of the LR at heading stage is not available.

Discussion

The activities of SDH and POD in both SRA and LR showed patterns of decrease and increase, respectively, with age, except for POD activity in SRA at heading stage (Figs. 2 and 4). Judging from this general trend, the use of both enzyme activities as the indicator of senescence seemed to be reasonable in the rice seminal root system as proposed by Yoshida and Takahashi[21].

However, detailed timecourse analysis of both enzyme activities and their comparison between SRA and LR in this present experiment revealed some problems in the use of both enzyme activities as indicators of senescence. Firstly, SDH activity could be used as an indicator only when the root system is very young because activity decreased rapidly in a few DAS and thereafter maintained almost the same value as shown in Fig. 2.

Secondly, the strict use of POD activity as an indicator of pure senescence was questioned. According to their proposed idea, the result of high POD activity in LR compared with that in SRA (Fig. 4) could be interpreted in such a way that the senescence of the LR progressed more rapidly than that of the SRA, indicating that the decrease of physiological activity in LR, also. However, such interpretation was incompatible with the result of our previous study[13] in which LR always maintained higher concentrations of carbon and nitrogen than SRA. In contast to the idea of Yoshida and Takahashi, Birecka et al.[1] speculated that, since POD had the ability to eliminate the phytotoxic hydrogen peroxide whose production in cells increases with senescence, an increase in POD activity would represent an induced protective reaction, which may delay the senescence. According to this idea, we can also interpret our present result in such a way that higher POD activity in LR would be related to some extent in the protective mechanism of rice root system against senescence. This interpretation corresponds well to our previous result mentioned above. Furthermore, an elevated POD activity in LR synchronized with the onset of development of 2nd order LR agrees with the interpretation. Therefore, the results of our previous and present studies strongly suggested that POD activity would represent not only the process of senescence but also an induced protective reaction, which supports the speculation by Birecka et al. Further study is needed to clarify the interrelationship among POD activity, senescence and protective reaction in rice root system.

Although several researchers have so far attempted to evaluate by visual inspection the logevity of seminal root systems in gramineous plants grown under upland soil conditions[2,6,18], there were many discrepancies in the data, which often differed depending on the species, methods and/or growth conditions of plants examined. Indeed, it is very difficult to estimate how long each seminal root system component stays alive and maintained its physiological activity because the senescence patterns are different among the root system components and the heterogeneous distribution of soil environmental factors further complicate the pattern.

Using the method of nuclear staining with acridine orange, Fusseder[2] observed that the senescence of cortical cells in the 1st and 2nd order lateral roots of corn plant grown in soil commenced near the root tip in a few days after their emergence and advanced toward the basal region of the root. He also pointed out that, at the late grain filling stage, all of the lateral roots along the main root axis exhibited progressed senescence, although stainable nuclei were seen in the root tissues of the basal part of the 1st and 2nd order lateral roots.

In this study, at the heading stage when the oldest LR were 84 days-old, about 8% of the 1st order LR which initiated on the basal 11 cm-portion of the seminal root axis were still stained in red partially by TTC reduction assay (Fig. 3). Moreover, at heading stage, it was observed that the 1st order LR which negatively reacted to TTC reduction assay was still firmly attached to the SRA. If these LR were completely dead, most of them must have decayed and sloughed off, and as a result, the number of 1st order LR must have decreased markedly as senescence of the seminal root system progressed. However, such phenomenon was not recognized in this study. Namely,

the number of the 1st order LR decreased only 14% from 154 at 14 DAS to 131 at heading stage. Consequently, we interpret these results in such a way that the LR of rice seminal root system grown in submerged woil conditions has a considerably long life-span, and the older parts of rice seminal root system examined are still alive even at heading stage.

We concluded from this study that in rice seminal root system, the senescence of SRA precede that of LR, and LR continuously maintain higher physiological activity than SRA. Furthermore, rice seminal root system was not dead but still alive up to heading stage. It is of great interest to examine how such physiological activities are related to root function, which is subject to further study.

Acknowledgment

This study was supported by a grant from the Japanese Ministry of Education, Science, Sports and Culture (Grant No. 04454043).

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* In Japanese with English summary.
** In Japanese with English abstract.
*** Translated from Japanese by the present authors.