Diagnostic Methods for the Measurement Of Root Activity in Rice Plant

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Introduction

Even though many established diagnostic techniques in relation to the soil and crop growth are prevalent to aid the farmers in adapting the proper method of cultivation, yet special attention has not been paid to the diagnostic study of root functions.

This necessitates the maintainance of the physiological activity of the root in rice plant at a high level by adaption of suitable techniques such as deep-tillage, soil admixturing, subsoil drainage, liberal application of farmyard manures and management practices such as intermittent irrigation and heavy temporary drainage.

This report intends to introduce the diagnostic methods for the measurement of root activity in rice plant from a practical standpoint.

Root system formation in rice plant

It is hoped that prior introduction of the process of development of the rice root system would be beneficial in understanding the diagnostic methods that are described in this paper.

Fujii (1961)[2] observed in his study of root shoot relationship that the root of a certain node and the upper third leaf from the node emerged and elongated simultaneously and that high correlation between the growth of roots and leaves in successive node was maintained.

For example, when the fourth leaf on the main culm develops, the leaf of the first primary tiller and roots from the first node of the main culm would develop simultaneously. Similarly when the fifth leaf of the main culm develops, the first leaf of the second primary tiller, the second leaf of the first primary tiller and the root from the second node of the main culm would develop synchronously.

The profile of the root sphere in the paddy soil may differ with the growth stage. At the primordial initiation stage, it may be oblong while after that stage it may be ovoidal consisting of two parts; i.e. a thickly rooted oblong sphere at the peripheral layer of the soil and a more or less loosely rooted sphere at a deeper layer.

The first part is called "superficial roots" which emerge horizontally from the nodes at an angle of more than 90° and spread over into the upper layer of the soil, like a net having numerous branch roots. The second part is called "crown roots" which emerge and spread into the deeper layers of the soil (Fig. 1).

The differential and developmental patterns of tissues in the crown roots of rice plant are summarized diagrammatically in Fig. 2.[5]

Kawata (1956)[4] found that shoots of rice plants could be designated as "shoot units", each with an apical leaf, a basal bud and upper and lower root zones (see Fig. 3). The upper and lower primary roots appearing on a shoot unit emerge essentially at the same time.

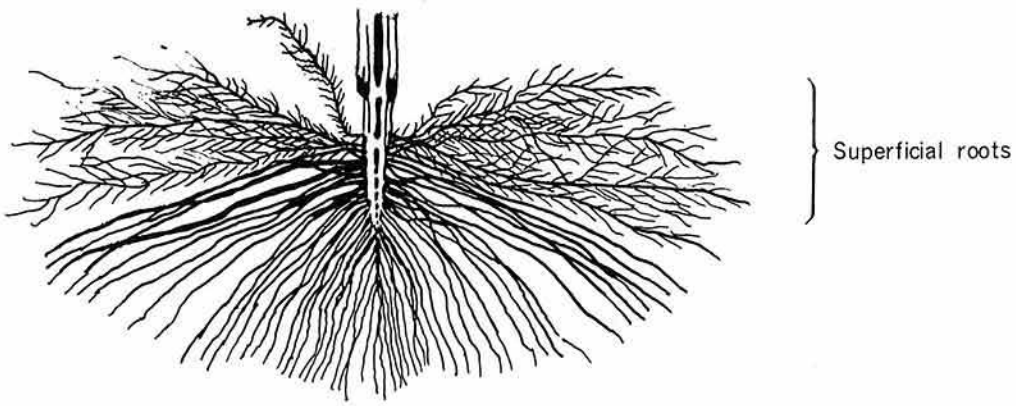


Fig. 1. Root system in rice plant.

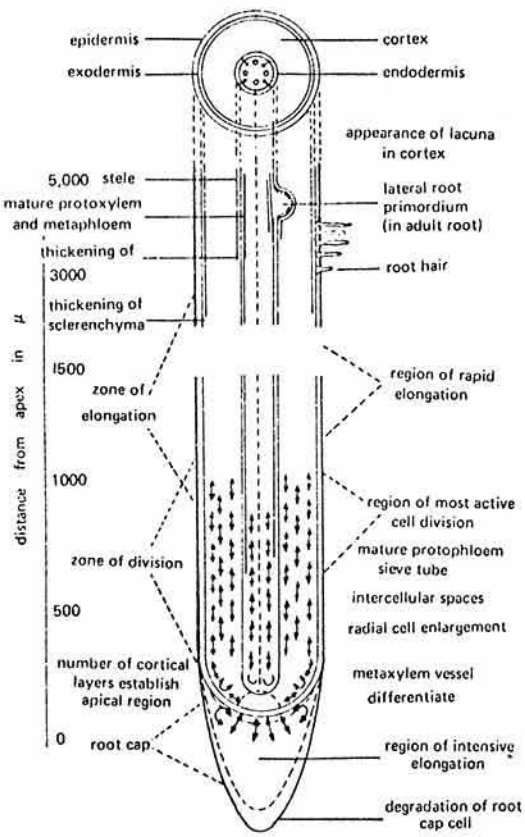


Fig. 2. Differential and development patterns of tissue in crown roots of rice plant.

The lower (basal) roots of a shoot unit are usually larger in diameter than the upper (apical) ones.

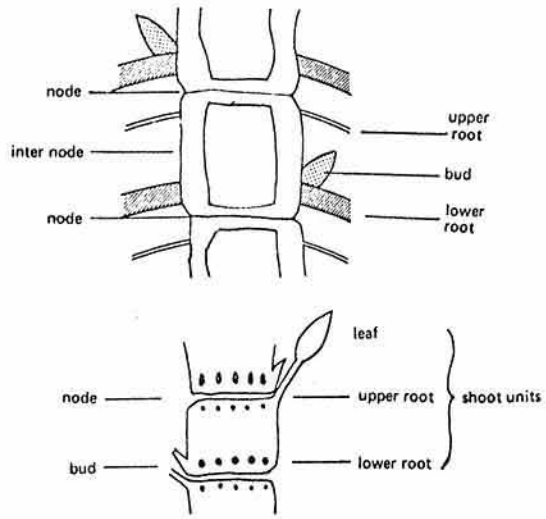


Fig. 3. Diagram of "short units" in rice plant.

Methods for the observation of root functions

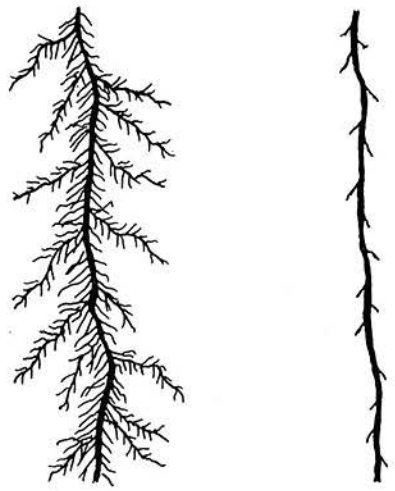
Even though the usage of these methods involve certain difficulties such as the changes brought about by variations in the soil and growth stage, yet they have the advantages of simplicity and rapidity.

Only general descriptions of these methods are discussed in this paper and their practical usage may need modifications depending on the individual circumstances.

1) Lateral roots and root hairs formation

The formation and development of lateral

roots and root hairs have been found to be more under conditions favorable for the oxidative condition of the soil. Thus, observation of the development of these root parts would be helpful in judging the soil condition under which they have grown (Fig. 4).



Oxidative condition Reductive condition

Fig. 4. Development of lateral roots in rice plant.

2) Discoloration of roots

Generally, under submerged soil condition, the ferric iron present in the soil is changed into a ferrous state owing to the reduced condition of the soil. However, the oxidizing activity of the rice root maintains a certain zone around its periphery in oxidative condition, reconverting the ferrous iron back into the ferric state.

In the early stage of the growth, it is possible for the root to maintain its whiteness due to its higher ability to oxidize and reconvert the ferrous iron present within this zone. As the crop growth advances, more and more ferrous iron gets fixed to the root surface changing its color from light brown to reddish brown because of the decrease in its oxidizing activity.

Therefore, under circumstances wherein the soil is highly reductive, even direct penetration of ferrous iron into the root tissues may occur, changing the root color to black or greyish white due to the formation of ferrous sulphide.

Thus, observation of the discoloration of roots would be helpful in judging both the oxidizing activity of the roots as well as the soil condition under which they have grown (Fig. 5).

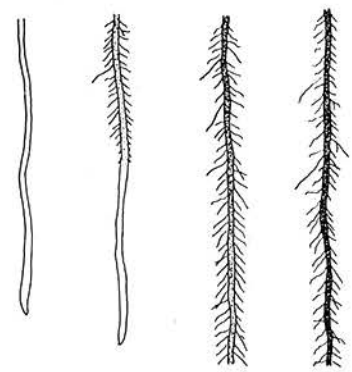


Fig. 5. Discoloration of roots in rice plant.

3) Occurrence of abnormal roots

Under severe condition of soil reduction occurrence of abnormal roots such as "lion tail"

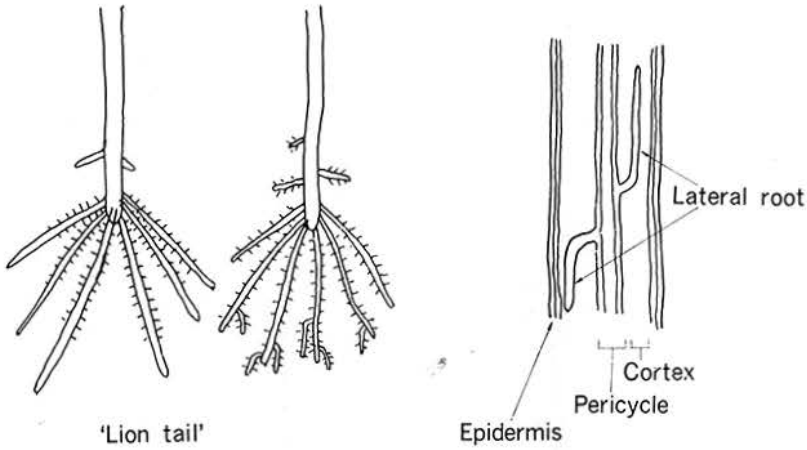


Fig. 6. Abnormal roots in rice plant.

may be noticed on account of the formation of toxic levels of hydrogen sulphide, organic acids or ferrous iron in the soil.[7]

Also, excessive soil nitrogen has been found to be associated with the formation of such "lion tail".

High concentration of hydrogen sulphide might cause the roots that emerge from the pericycle to grow into its own cortical tissue to make them abnormal (Fig. 6).

4) Development of the ventilating system

Generally, the rice plant has a self-ventilating system by which oxygen is transferable from the shoot to the root and the degree of development of this system will give an indication of the tolerance of the plant to reductive soil condition. It seems that the capacity of this ventilating system is dependent upon the root diameter.

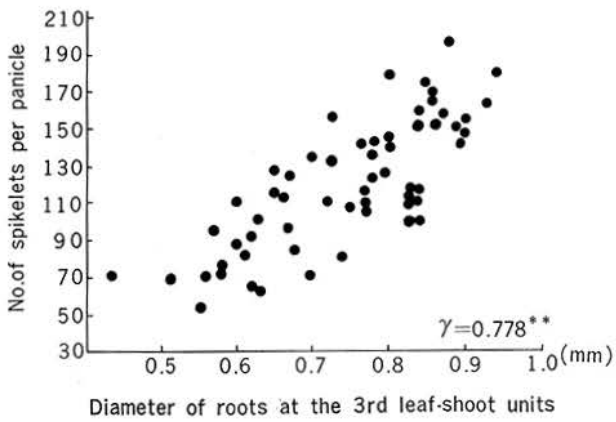


Fig. 7. Relation between the number of spikelets per panicle and the diameter of roots in rice plant.

Root diameter is also found to be correlated to the culm thickness and number of spikelets per panicle (Fig. 7). Thus, the selection of the panicle weight type of varieties may be more beneficial for growing under such reduced soil conditions.

5) Aging of the roots

In the system of classification of roots as old and young based on their points of nodal origin, more differences exist between the old and young roots, the more has been the unsatisfactory conditions of the soil.

Likewise, it is also true, that under very favorable soil conditions, it will be more difficult to distinguish the old and young roots because under the favorable soil conditions the senescence of old roots gets delayed. On the other hand, when the soil conditions have been unfavorable, senescence of the older roots occur relatively quick and discoloration of the root takes place due to the resulting loss in root activity.[6]

Thus, the importance of root activity depends mostly on the activity of older roots since in younger roots, much variations might not occur. Therefore, when abnormalities in

the development of roots are noticed, the correct judgment of the stage of occurrence of such abnormality by knowing the nodal origin of such roots, will help in deciding the stage at which the soil or environmental features have not been favorable to the root growth.

Methods for the determination of root function

Even though the root activity could be effectively measured by its respiratory rate, yet the usage of apparatus such as Warburg's manometer is limited due to the complicated nature of its operation and the limited number of samples that could be handled at any time.

However, the adaptation of simple methods such as using α-naphthylamine[8] and esculin for the measurement of oxidizing activity, or the usage of T.T.C. to measure the reducing activity of roots[1] have made this easy.

The oxidizing activity of roots can be evidenced by measuring the oxidation of the α-naphthylamine by roots. In case where the nutrient absorption by roots is inhibited, the oxidizing activity of roots is usually very low. The α-naphthylamine oxidation is related to the rate of respiration. As a result, a parallel relationship is, in most cases, observed between the respiratory rate and the α-naphthylamine oxidizing activity (Fig. 8). Usage of α-naphthylamine for the determination of the oxidizing activity of roots is described below.

1) Sampling and classification technique

By using steel plates, a block of soil is lifted from the sampled area and is separated either into layers of different depths (generally upper 5 cm and the remaining lower layer)[3] or by the point of root origin (generally as roots originating from the upper three nodes and the remaining as lower nodal roots)[6] (Fig. 9).

2) Determination technique

Reagents:

(1) 100-ppm α-naphthylamine solution (stock solution)

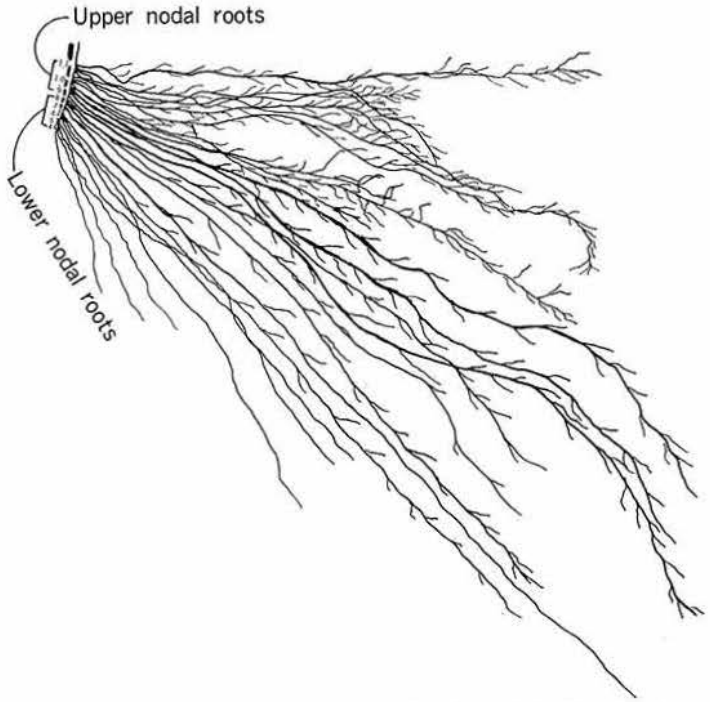


Fig. 8. Classification of roots by the point of root orign.

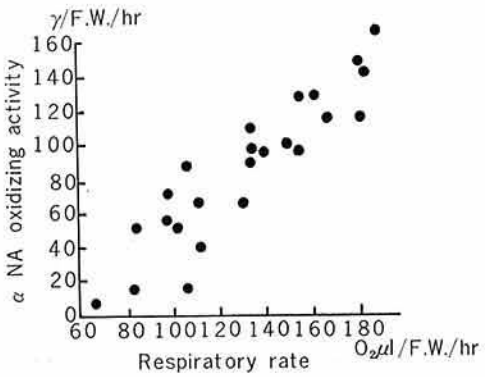


Fig. 9. Relation between the respiratory rate in roots and the α-naphthylamine oxidizing activity by roots in rice plant.

100 mg of α-naphthylamine is dissolved in water, and made up to 1 liter (α-naphthylamine is very difficult to dissolve and hence the mixture is kept on a shaker over night). Before use, this stock solution is diluted 5 times with water to get 20-ppm solution.

(2) 1% sulphanilic acid solution

1 g of sulphanilic acid is dissolved in 100 ml of 30% acetic acid.

(3) 100-ppm sodium nitrite solution

100-mg sodium nitrite is dissolved in water and made up to a liter.

Procedure:

The classified root samples are washed carefully in water, cut into segments of 1–2 cm and mixed thoroughly. After squeezing the excess water, 1–2 g of this mixed sample is weighed and transferred into a conical flask containing 50 ml of 20-ppm α-naphthylamine solution and this flask is incubated for 2–3 hours under continuous shaking.

2 ml portion of the α-naphthylamine is pipetted out before and after incubation into a graduated test tube diluted with 10 ml of water. 1 ml of 1% sulphanilic acid and 1 ml of 100-ppm sodium nitrite solutions are added and the mixture made up to 20 ml with water. After 30–60 minutes spectrometric determination using 500 mμ is made of the α-naphthylamine concentration.

3) Calculations

By using different concentrations of α-naphthylamine in 20 ml of made up solutions, a calibration curve is obtained and the α-naphthylamine concentration in the root solution before and after incubation is measured using this calibration curve.

From this result, the quantity of α-naphthylamine oxidized is calculated in γ per g of fresh weight of roots per hour.

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