DETERMINATION OF THE FREE RADICAL CONTENT OF RICE DURING FLOWERING BY THE ESR TECHNIQUE

J. J. CHINOY, O. P. SAXENA, I. C. DAVE, K. GURUMURTI, R. B. PANDYA AND P. G. ABRAHAM

Botany Department, University School of Sciences, Gujarat University, Ahmedabad-9, India

CONSIDERABLE evidence has accumulated by now to highlight the importance of free radicals in the process of energy transfer not only in pure chemical reactions but also in biological processes of both the plant and the animal.[1] Commoner and his associates[2] have done pioneering work in experimentally establishing the participation of free radicals in one-electron transfer by study of electron spin resonance in biological materials. Commoner[3] has also confirmed the photosynthetic free radical component (II) in isolated chloroplasts as well as in whole living algæ. Gurevich[4] reported that isolated wheat and corn embryos, etiolated corn seedlings and the embryonic parts of corn leaves all contained a special enzyme, peroxidase, which catalyzed the production of the free radical of ascorbic acid, called monodehydroascorbic acid.

Enhancement in metabolic processes as well as an increase in the productivity of cucumber and tomato plants raised from irradiated seeds was found to be due to the increased concentration of free radicals.[5]

It has been shown in this laboratory that higher ascorbic acid (AA) content as well as its utilization (AAU) synchronize with a change in the shoot apex of cereals from the vegetative to the reproductive state.[6] The activity of the special peroxidase, which produced the free radical of AA, was also found to be at a higher level during the period of reproductive differentiation[7] compared to that prevailing during the vegetative phase indicating enhanced production of AA-free radical.

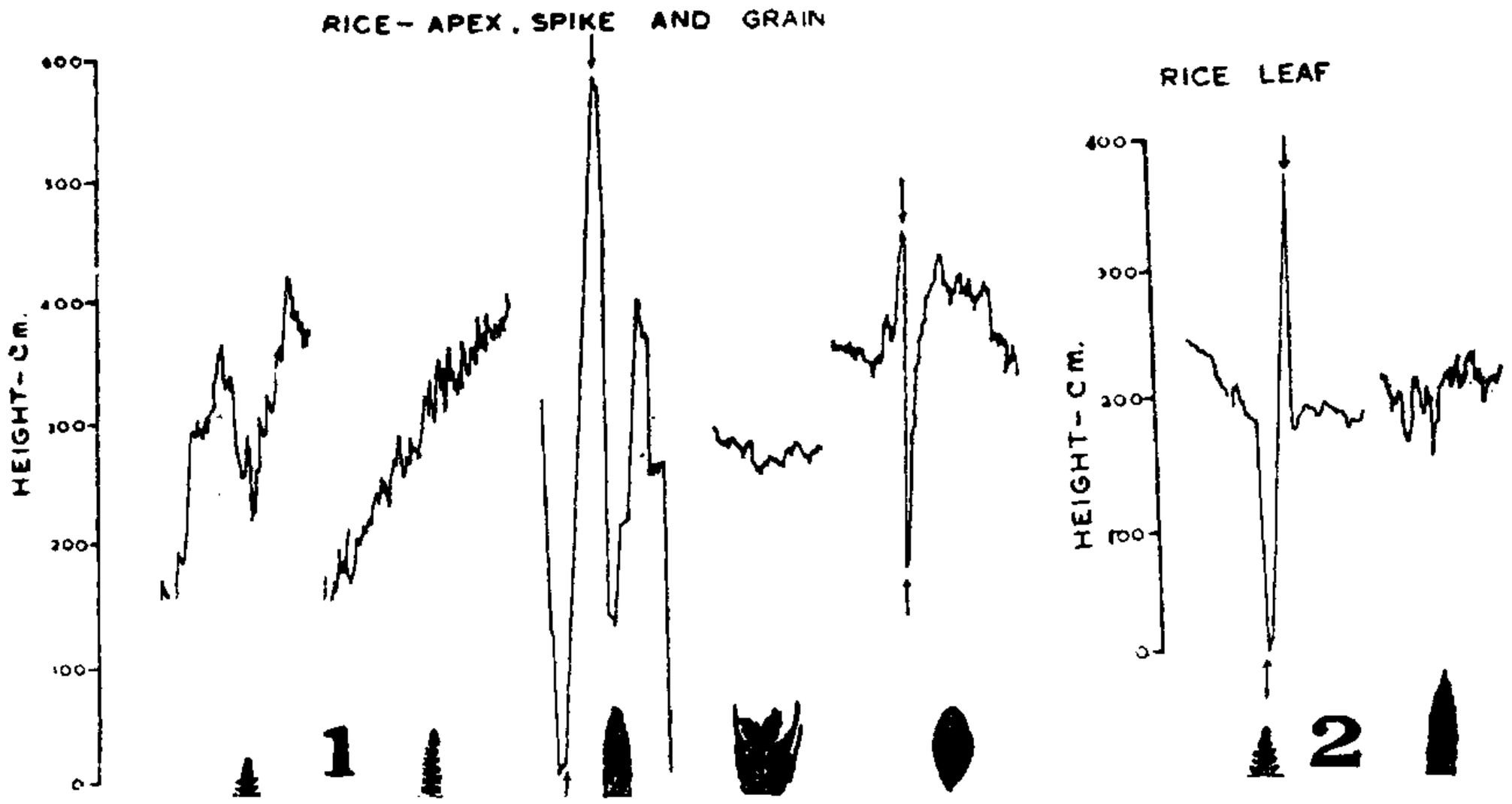
It was therefore considered of interest to confirm the presence of free radicals which were previously determined biochemically by the ESR technique during the periods of vegetative and reproductive differentiation of some plants. The data for free radicals are presented here only for rice in terms of arbitrary units—height of the signal in cm./g. fr. wt. for the sake of simplicity of comparison for various growth and developmental stages. The detailed quantitative work on this aspect will be reported elsewhere.

Vegetative shoot apex, primordial spike, floral organs and developing grain as well as leaves at the above-mentioned stages of rice var. HR-5 were collected from growing crop. The tissue was weighed immediately and transferred to the moist filter-paper in blackened petri dish so as to check the initiation of senescence and light-induced ESR signals. The intact tissues were dissected just before their transfer to the sample tube in order to prevent increase in peroxidase activity in excised tissues. The samples were screened for free radicals using a modified version of a Varian 100 Kc electron spin resonance spectrometer. The samples were weighed and pressed gently between the two filter-papers and then the samples were transferred to the sample tube to be fixed in a sample cavity in a Dewar fiask containing liquid nitrogen at 77° K. (– 196° C). The data (height of the signal in cm.) presented in Figs. 1 and 2 have been calculated for a gram fresh weight of tissue and to constant values of the 100 Kc field modulation and amplifier gain.

The free radical content in the shoot apex slowly increases during the period of vegetative differentiation. However, at the time of transformation of the shoot apex from vegetative to reproductive phase, a many-fold increase in the production of free radicals is clearly seen. The free radical content decreases in the primordial spike. A sharp rise in the free radical content is recorded once again during the embryo differentiation of developing grains (Fig. 1).

The free radical content of the leaf is higher during the period of vegetative differentiation compared to that found in the same organ during the period of reproductive differentiation (Fig. 2).

The g-value of the observed electron spin resonance is close to the free electron value of 2·0023 and one can be reasonably certain that the free radicals are organic in nature. On the basis of the fact that AA, AAU and the FR-peroxidase increased considerably during the period of reproductive differentiation one of us has postulated that the enhanced



FIGS. 1–2. Fig. 1. Free radical content in shoot apex, spike and developing kernels of rice. Fig. 2. Free radical content in leaf of rice.

concentration of free radical of AA (and perhaps that of other organic substances) during the period of reproductive differentiation supplied enhanced electronic energy for the highly activated biosynthesis of DNA, RNA, proteins, enzymes and other cell constituents required for accelerated cell division and laying down of growth centres during the process of flowering. The present work confirms by EPR measurements the presence of increased free radical content of rice during the flowering process.

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