Automorphogenesis of Plant Seedlings under Simulated Microgravity on a 3-D Clinostat

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Abstract We developed a three-dimensional (3-D) clinostat equipped with two motors and studied the changes in different growth processes of higher plants under this condition. On the clinostat, morphogenesis of seedlings changed dramatically. Shoots and roots exhibited a spontaneous curvature as well as an altered growth direction. The curvature occurred at different portions of seedlings. Maize shoots exhibited the curvature in the transition zone connecting roots and mesocotyls, coleoptile nodes, and coleoptiles. Maize roots bent in the most basal region, the region between the mature and the elongating zone, and the elongating zone. The curvature became larger with their growth. Such an automorphogenesis may play an important role in regulation of life cycle of higher plants under a microgravity environment.

Key words: automorphogenesis, 3-D clinostat, growth, maize, *Zea mays* L.

Introduction

The life of higher plants is dependent on the gravity of 1 g on earth. In order to understand the role of gravity in regulation of plant life cycle, it is required to examine the changes in each process under microgravity conditions. For this purpose, we developed a new clinostat equipped with two rotation axes placed at right angle (Hoson et al. 1992a). Of different growth and developmental processes that characterize vegetative growth phase of higher plants, morphogenesis was most strongly influenced by the clinostat rotation (Hoson et al. 1993). The details of such a spontaneous morphogenesis, "automorphogenesis" (Pfeffer 1904), in maize seedlings will be described.

Materials and Methods

The 3-D clinostat was designed and constructed as reported previously (Hoson et al. 1992a). The main power of rotation was provided by two geared stepping motors. The sample stage and the illumination apparatus were attached to the opposite sides of a supporting frame which was rotated by one motor (Fig. 1). The frame was attached to the other frame which was further rotated perpendicularly about an axis parallel to the ground by the second motor. Germinated caryopses or seeds were attached to the sample holder fitted in a cylindrical culture container which was fixed to the sample stage. They are continuously rotated three-dimensionally by changing the rate of rotation of two motors at random. The rotation of

Figure 1 The clinostat apparatus. L, light; M, motor; S, sample stage; SR, slip-ring.

motors was observed with encoders. Electrical connections to motors, illuminations, encoders, and a video terminal were made via sliprings. The onset, the rate, and the duration of rotation of motors were controlled with a personal computer.

Results

The clinostat rotation did not influence the growth rate of shoots or roots. In contrast, the direction of growth of plant organs clearly changed on the clinostat. On the ground, shoots generally grew upward parallel to the gravity vector, although a slight abaxial (away from the seeds or caryopses) curvature was observed in some species. On the clinostat, shoots showed either an adaxial (toward the seeds or caryopses) or an abaxial curvature depending on species (Fig. 2). Roots grew

Figure 2 Seedlings grown on the 3-D clinostat. Roots of maize, pea, and azuki bean grew in random directions from the early stage of growth.

downward on the ground. In most species, roots elongated along the direction of the tip of primordia in the early stage of growth and then in a random fashion (Fig. 2). The duration of the former period was variable among species.

The automorphogenesis of plant seedlings on the clinostat was a complex phenomenon composed of various elements. The process was analyzed in detail in maize (*Zea mays* L.) seedlings. The curvature of maize shoots was found to consist of three components; 1) the bending in the transition zone connecting roots and mesocotyls, 2) the bending at coleoptile node, and 3) the curvature of coleoptiles (Fig. 3). Control seedlings showed a slight abaxial bending in the transition zone. Clinostat rotation stimulated such a bending. On the contrary, shoots bent adaxially toward the caryopses at coleoptile node between mesocotyls and coleoptiles. The angle was small in the control, while it was larger on the clinostat. Control coleoptiles elongated almost straight, whereas most of coleoptiles bent abaxially on the clinostat. The curvature in three regions

Figure 3 Typical example of maize shoots grown on the ground or on the 3-D clinostat for 3 days.

became larger with shoot growth. In a front view of embryos, an unequal distribution of shoot angle was not observed. Thus, maize shoots exhibited a spontaneous curvature on the clinostat in the direction explained by the presence of dorsiventrality.

Maize roots, which consist of only one organ, were also found to exhibit a spontaneous curvature in three different portions; 1) the basal region just protruding from coleorhizae, 2) the region between the mature and the elongating zone, and 3) the

Figure 4 Typical example of maize roots grown on the ground or on the 3-D clinostat for 1 day.

elongating zone, several mm from the tip (Fig. 4). Even control roots showed some degree of the curvature in all regions. The curvature in region 1 was most prominent. The clinostat rotation greatly stimulated the curvature and caused an increase in the dispersion of angle. In regions 2 and 3, the distribution of control roots was rather concentrated around 0 degree as compared with region 1. The curvature in these regions was also stimulated by the clinostat rotation. Growth direction of roots did not exhibit a clear dorsiventrality. An unequal distribution of root angle was also not observed in a front view of embryos. In most control roots, the curvature in region 2 or 3 occurred in a direction opposed to that in region 1. Thus, roots as a whole grew downward in parallel to the gravity vector on the ground. On the contrary, the curvature occurred in random directions in all regions on the clinostat.

Discussion

Under simulated microgravity conditions on a 3-D clinostat, maize shoots exhibited a curvature in three portions in the direction explained by the presence of dorsiventrality, while maize roots bent in three regions in random directions. A slight abaxial bending at the transition zone connecting roots and mesocotyls and an adaxial curvature at coleoptile nodes were also detected in maize shoots grown on the ground. Control roots also exhibited some degree of curvature in the three regions. Thus, a characteristic curvature in different regions of seedlings observed on the clinostat may be derived from inherent properties of plants, which would be modified by the gravity vector on the ground.

On the 3-D clinostat, the amyloplasts are distributed at random in statocytes of cress roots (Buchen et al. 1993) as observed in the space experiment

(Volkman et al. 1986). Such changes in the ultrastructure of statocytes appear to represent the influence of simulated microgravity on gravity sensing mechanisms. Some differences were detected in the mechanical properties of the cell wall between the concave and the convex side of maize shoots exhibiting a spontaneous curvature (Hoson et al. 1992b). The further study of events which connect these two phenomena will clarify the role of gravity in morphogenesis of higher plants.

In the short-term experiments in the dark, growth correlation or the translocation of sugars was not influenced by the clinostat rotation, in spite of clear changes in morphogenesis (Hoson et al. 1993). However, normal morphogenesis of seedlings appears to be prerequisite for long-term vegetative growth. The automorphogenesis should play a principal role in regulation of plant life cycle under a microgravity environment.

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