On the Multicellular Protuberances Developed on Barley Leaves under Aseptic Condition

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This paper represents that protuberances are produced by high auxin levels on foliage leaves of barley. They were seen on barley seedlings, cultured in abundant hormonal circumstances. Such protuberances indicate an interesting problem with relation to hormonal regulation in the outgrowth of epidermis in grass leaves.

**Material and Method**

Barley seeds (*Hordeum vulgare*, variety: Musashinomugi) were employed in this experiment. Surface sterilized seeds were seeded and cultured on the modified Murashige and Skoog’s basal medium in which growth substances and sucrose had been added according to the previously described method*, which induce callus tissues from the rice embryos. Culture bottles, 100 ml Erlenmeyer flasks containing the barley seeds, were maintained at 30°C under both light and dark conditions.

**Experimental Results**

Several weeks after the start of the culture, the protuberances were produced on the foliage leaves of the seedlings cultured aseptically. They were frequently formed in the medium supplemented with 2, 4-D (2, 4-dichlorophenoxyacetic acid: $3 \times 10^{-5} M$) and yeast extract (5 g/l) and even in the media containing $10^{-5} M$ 2, 4-D with or without yeast extract and $10^{-5} M$ 2, 4-D plus 0.5 ppm kinetin.

An excised leaf producing the protuberances was fixed by the fixative (FAA) after 36 days of cultivation. Fig. 1 shows the apical region of leaf observed. The protuberance on leaf surface is indicated in fig. 2. As shown in the figures (1, 2), the protuberances are 5 mm or more in length. They emerged downwards from the abaxial surface of laminae and occasionally from the adaxial surface or the leaf margin.

They were stained in Delafield’s hematoxylin, squashed in glycerin and observed under the microscope. In fig. 3, the terminal tip is represented photomicrographically. It is concluded that the protuberance is certainly a multicellular structure having apical meristem but not root-cap like tissue, and resembles leaf serration or ligule rather than root or trichome like structure. The structure may be leaf like, because of the presence of small vascular bundles and precocious stomata.

Comparable outgrowths were also occurred from coleoptile tips under the culture condition without hormonal treatment in the barley variety used in this experiment (figs. 4, 5). Thus it is assumed that the coleoptile tips have higher level of auxin, because the protuberances occurred from barley leaves under high level of auxin.

**Discussion**

Norstog* have indicated the occurrence of the epidermal hairs identical to leaf hairs in the scutellum and coleoptile of barley by the supplements

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of auxin and other hormonal substances. It has been well known that dorsal teeth are produced on the keel of leaves in Aloe mitriformis\(^5\) and also buds or bud-like outgrowths are formed on leaves in Begonia hispida\(^3\), B. phyllocladica\(^2,3\), B. rex, Bryophyllum crenatum and Streptocarpus wendlandii\(^4\) etc. Furthermore, it is peculiar interesting to note pinnate outgrowths of Eriocarpum leaves\(^1\), “Ryu” in Rohdea japonica\(^5\) and “Diplophyllie” in Alchemila\(^1\).

It has been reported that enhancement of budding by abscisic acid\(^6\) and cytokinin\(^1\) in Begonia leaves and by cytokinin\(^5\) in leaves of Crepis capillaris are resulted. But such protuberance as developed on the barley leaves by hormonal treatment, being indicated in this paper, is not reported yet, and hence it may be of great interest to investigate the hormonal regulation of differentiation in the epidermal system of monocotyledonous crops.

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**SUMMARY**

Protuberances were formed on the leaves of barley seedlings cultured under the aseptic conditions. They were caused by high concentrations of 2, 4-D in the abaxial and adaxial side of laminae and the leaf margin, indicating multicellular structure which have vascular bundles and stomata. These evidences are discussed comparing with the protuberance formed in the tips.
of barley coleoptiles, and further with several appendices of the leaves in other plants.

LITERATURE CITED


[和文摘要]

無菌条件下オオムギ葉から生じた多細胞性突起について

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オオムギ（品種ムサシノオムギ）種子を、ショ糖と寒天をくわえた Murashige-Skoog の培地に植え、無菌条件下で培養した。数週間後、葉の上に突起物が生じた。このものは 3×10^{-5} M の 2, 4-D と 5 g/l のイースト抽出物をくわえたとき、しばしば見られた、長さ 5 mm 程度のものもあり、葉身の背側から下方に向けて突出しているものが多かった。この突起は、多細胞により構成され、根冠状組織をむしろ、鋸歯または葉舌に似た形態をしています。子葉鞘の先端にも、よく似た形態の突起が見られた。

以上の結果をもとづき、作物の分化制御に関する植物ホルモンの作用性の面から、Alchemilla, Aloe, Begonia, Bryophyllum, Crepis, Erigeron, Rothea, Streptocarpus の葉上突起物について観察と比較考察した。