

## IAA, ABA AND GERMINATION OF SPRING BARLEY CARYOPSES DURING POST-HARVEST MATURATION

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**Changes in the content of indole-3-acetic acid (IAA) and abscisic acid (ABA) were investigated in the caryopses of three spring barley varieties (Alexis, Akcent and Rubin) shortly before harvest and during post-harvest maturation. Simultaneously, the values of the germination index and germinating energy were followed after harvest. The differences in endogenous contents of the phytohormones were determined in the caryopses of all three barley varieties which showed a decreasing pattern during post-harvest maturation while in contrast the values of the germinating energy and germination indices increased. Significant differences were observed between the varieties.**

**Key Words:** *Barley caryopses, maturation, dormancy, germination, ABA, IAA.*

### INTRODUCTION

Uneven germination of barley caryopses under optimal conditions in the post-harvest period is defined as dormancy. The depth (intensity) of dormancy and duration of the breaking of dormancy, i.e. post-harvest maturation, are responsible for considerable problems in malthouses at the beginning of the season. Post-harvest maturation of barley is a varietal characteristic and its duration is affected by the environmental conditions which prevailed at the time between fertilization and harvest maturation of the caryopses<sup>1,4</sup>. Nevertheless, dormancy of the barley caryopses is an important factor before the storage of harvested grains because it prevents viviparous germination.

The present knowledge of the physiology of seed germination outlines some factors promoting or suppressing germination and the significant role in these processes played by phytohormones<sup>5</sup>, especially gibberellins, abscisic acid (ABA) and probably indole-3-acetic acid (IAA).

ABA plays an important part during maturation of the caryopses and dormancy<sup>1,2,5,4,2</sup>. Exogenously applied ABA inhibits the production of  $\alpha$ -amylase<sup>6</sup> and germination of isolated barley embryos<sup>3</sup>. The sensitivity of the embryos to exogenous application of ABA depends on the intensity of dormancy of the caryopses<sup>7</sup> but the role of IAA in dormancy and post-harvest maturation of the barley caryopses is not clear<sup>1</sup>.

A thorough understanding of the physiology of barley caryopses before and after harvest should give an insight into the changes inducing dormancy and also into changes which occur during post-harvest maturation. An understanding of these factors before and during post-harvest maturation and the germination differences between barley varieties could be utilized in the breeding of varieties and in the technological measures used to make malt.

### MATERIAL AND METHODS

#### *Plant material*

In 1995, the barley varieties Alexis (Breun, Germany), Akcent (Selgen, Czech Republic) and Rubin (Plant Select, Czech Republic) were grown on the premises of the State

Variety Testing Laboratory in Věrovany. The harvest was carried out on July 27, 1995 while samples of the caryopses for analyses were collected before the harvest on July 10 and July 24, 1995. The material was sorted and the portion above a 2.5 mm sieve was used for further analyses.

#### *Germination analysis*

The germination index and germinating energy<sup>11</sup> were determined under the following conditions. The caryopses were germinated on filter paper at 20°C. The germinated grains were counted and removed after 24, 48 and 72 h. The germinating energy was defined as the percentage of caryopses that germinated within 72 h. The germination index was calculated according to Riis and Bang-Olsen<sup>11</sup>.

#### *Sample preparation and IAA determination by GC*

A 20 g batch of the caryopses was overlaid by liquid nitrogen and homogenized for 1 min. The material was then overlaid by 150 ml of 96% methanol with 100 mg  $\cdot$  dm<sup>-3</sup> BHT (butylhydroxytoluol) (Sigma) and extracted at 4°C. After 24 h the sample was filtered and the homogenate was extracted again with 75 ml of 96% methanol with BHT. The same extraction was repeated once more and the filtrates were pooled and the volume adjusted to 250 ml by 96% methanol with BHT. 1 ml of the extract was loaded on Sep-pak C18 column (Tessek) equilibrated with 5 ml of methanol. The purified sample was derivatized with hexafluorobutyrylimidazole in pyridine. Chromatographic analysis was carried out on Fisons GC 8000 gas chromatograph equipped with an electron capture detector (ECD) by on-column injection. A nonpolar column (100% dimethylsiloxan) quartz capillary Spira K15 (Lachema), length 25 m, internal diameter 0.32 mm, film thickness 0.5  $\mu$ m was used. The total IAA content is reported in ng  $\cdot$  g<sup>-1</sup> of fresh matter.

#### *Sample preparation and ABA determination by Radio Immuno Assay (RIA)*

A 1 g batch of the caryopses was overlaid with liquid nitrogen and stored in a glass vial at -24°C until analysed by the method of Quarrie and Galfre<sup>10</sup>. The samples of the caryopses were homogenized in water and shaken in the dark at 4°C for 12 h, centrifuged and four 50  $\mu$ l aliquots of the supernatant taken for analysis. RIA analysis was carried out using a MAC 252 monoclonal antibody (Monoclonal Antibody Centre, Cambridge, UK) to determine (+)-S-ABA and <sup>3</sup>H-ABA. Radioactivity was measured on a Packard 2000



