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IAA DETERMINATION IN THE KERNELS OF FOUR LINES OF CORN AND OF THEIR HYBRIDS

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Abstract—The variation of concentration of free β-indolacetic acid (IAA) has been studied in the corn kernels of different inbred lines and their single and double crosses, at the early "milk" stage and at the stage of advanced ripeness. IAA was separated by thin-layer chromatography from the plant ethanolic extract. IAA content was determined by absorptivity measurement of eluates in u.v. light. A large decrease of IAA concentration was recorded as the kernels aged, and the single crosses W79A × W75 and 131B × 65B show a higher content compared with the respective parents. The amounts are smaller in double cross.

INTRODUCTION

FEW researches have been carried out on the variations due to hybridation in the concentration of constituents having a notable physiological effect. Richey and Dawson,[1] referring to the possibility of breeding corn with higher niacin concentration, established that for single- and double-cross hybrids, the concentration tends to be intermediate between that of the parents. Cherry and his co-workers[2] determined the content of acid-soluble nucleotides and of RNA in different corn inbred lines and of their hybrids. Zieserl and Hageman[3] studied the effect of genetic composition on the nitrate-reductase activity and soluble protein and nitrate contents in corn.

Brunson and Quackenbush[4] have compared the provitamin A content of corn inbred lines and of their single crosses. Alston and Hempel[5] have established that for specific flavonoids, the principle of cumulative inheritance in the hybrids of *Baptisia leucantha* × *B. Sphaerocarpa*, is exemplified.

In this paper the variations of free β-indolacetic acid (IAA) concentration in the corn kernels of different inbred lines and in their single and double crosses, at the early "milk" stage and at the stage of advanced ripeness, have been examined. IAA has been considered because of its large physiological effects.[6]

The fact that hybrid corns represent the most important application of genetics to agriculture[7,8] and consideration of the above-mentioned results[1–4] coupled with the fact that corn kernels, especially at the milk stage, are a rich source of free IAA[9] made them ideal experimental material.

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RESULTS AND DISCUSSION

IAA concentrations in the kernels of inbred lines, of the single and the double crosses examined, at early milk stage and at the stage of advanced ripeness, are shown in Table 1. The data reported in Table 1, regarding IAA concentrations in corn kernels at early milk stage, are of the same order as those given by Haagen-Smit *et al.*[9] The large decrease of IAA concentration passing from early milk stage to advanced ripeness, agrees with results of other workers.[6]

TABLE 1. IAA CONCENTRATION IN THE CORN KERNELS OF FOUR LINES AND OF THEIR HYBRIDS

	mg/100 g of dry matter	
	Milk stage	Advanced ripeness
131B	5·98	0·78
65B	3·47	0·78
131B × 65B	7 58	0 94
W79A	4·17	0 31
W75	3·77	0·20
W79A × W75	5·76	0 43
(W79A × W75) × (131B × 65B)	5·45	0 46

The single crosses W79A × W75 and 131B × 65B show an IAA concentration higher than the respective inbred lines; this is more evident when the kernels are examined at early milk stage; the differences at the stage of advanced ripeness are less easily seen.

However, the influence of inbred lines is clear in hybrids. From the two American dent inbreds having a mean IAA content of 3·97 mg/100 g of dry matter we obtain the hybrid (W79A × W75) with a content of 5·76 mg/100 g; from the two Italian flint inbred lines having a mean content of 4·72 mg/100 g of dry matter we obtain the hybrid (131B × 65B) with a content of 7·58 mg/100 g of dry matter (Table 2).

TABLE 2. COMPARISON BETWEEN RELATIVE CONCENTRATIONS IN THE KERNELS OF THE FOUR INBREDS AND THEIR HYBRIDS AT EARLY MILK STAGE

	IAA content (mg/100 g of dry matter)
131B	5·98
65B	3·47
Average of the two inbreds	4·72
131B × 65B	7·58
W79A	4·17
W75	3·77
Average of the two inbreds	3·97
W79A × W75	5·76
Average of the four inbreds	4·35
(W79A × W75) (131B × 65B)	5·45

Hybrid vigour, or heterosis, is expressed, according to Sprague,[10] when the F_1 hybrid exceeds both inbreds in total growth or in other measurable attributes. Even though hybrid vigour has been known for more than a century there is no theory which can explain the phenomenon. It seems possible that heterosis may be due to the complementary effect of different active substances from the physiological point of view, coming from inbred lines.[11] IAA concentration in kernels, together with other physiological and biochemical properties, might thus be a determinant of the hybrid vigour. In fact, experiments carried out by Key and Shannon[12] demonstrate that addition of IAA to plant cells causes a stimulation of RNA and protein synthesis. Cherry *et al.*[2] showed that a corn single-cross (WF9 × M14) had not only a higher RNA content, but also synthesizes RNA much faster than do both inbred lines. We suggest higher amounts of IAA in the kernels of single crosses are responsible for the results obtained by Cherry *et al.*

The data shown in Table 2 indicate that in the corn kernels at early milk stage heterosis reaches the maximum in the single crosses (the values for both hybrids exceed the average of the parental lines by about 50 per cent). In the double cross, the hybrid vigour is lower; in fact the value found in the double cross for IAA is only 25 per cent higher than the average of the four inbred lines.

EXPERIMENTAL

Plant Material

The dent corn American inbred lines, W79A and W75, are the parental lines of the single-cross seed parent of the diffused trading Wisconsin 270; the two flint corn inbred lines, obtained at the Institute of Agronomy of Perugia University, from an Umbrian corn population, through nine generations of self-fertilization and selection; these inbreds are indicated as 131B and 65B. The two single crosses resulting from the combinations W79A × W75 and 131B × 65B (the first line is the seed parent, the second one is the pollinator); the double cross resulting from the combination of the two above-indicated single crosses (known commercially as Etruria 280).

The material to be analysed (ears in different stages of ripeness) was picked as follows: W79A, from an isolated field in S. Costanzo, where the single cross W79A × W75 was produced; W79A was emasculated to allow the crossing; W75, from the same field of W79A; W75 was the pollinator. 131B, from an isolated field in Pallotta, where the single cross 131B × 65B was produced; 131B was emasculated to allow the crossing. 65B, from the same field of 131B; 65B was the pollinator. The single crosses, from an isolated field in Papiano, where the commercial double-cross Etruria 280 was produced; the W79A × W75 was the single-cross seed parent, while 131B × 65B was the pollinator. The double-cross Etruria 280, from a commercial field in Monte Melino. All the above-mentioned fields are situated in the hills near Perugia. In the different fields the cultural practices have been exactly the same: summer ploughing; pre-sowing manuring with 120 kg/ha of P_2O_5 as mineral superphosphate; manuring with 100 kg/ha of nitrogen as urea and ammonium nitrate three to four times during corn growth; two cultivations for weed control; irrigation after fertilization.

The soils had very similar characteristics, the clay content allows us to classify them as

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clay loam. The organic matter and the available P_2O_5 content is poor; the available K_2O content is very high. The sufficient identity of the soils excludes any possibility that the obtained results could have been influenced by nutritional effects.

An exceptional spring and summer compared with the regional climate was experienced in the year of the experiment; sowing was delayed about one month; a subsequent period of drought with high temperatures damaged corn crops mainly during fertilization. The ripening phase was, however, regular, perhaps due to irrigation.

Harvesting was always carried out at 7 p.m. Moisture content at the early milk stage was $75 \pm 3\%$ and for the stage of advanced ripeness $30 \pm 2\%$. The early milk stage was picked 17–20 days after self-fertilization. The samples were immediately put in polythene bags and brought to the Institute.

Extraction

The extraction method must ensure that chemical or enzymatic reactions do not modify the real content of free IAA actually present,[13,14] and consequently we have used ethanol at low temperature[15,16] (cf. Ref. 17). A 200 ml quantity of 95% ethanol was added to 20 g of the sample. After stirring, the solution was allowed to stand at −5° for 12 hr, centrifuged, and the residue washed three times with 50 ml of ethanol. The combined extracts are distilled at 35° *in vacuo* to a syrup.

The extract is adjusted to pH 8·5 with 5% $NaHCO_3$ and extracted three times with peroxide-free ether.[13] The ether fractions are discarded. The aqueous phase is adjusted to pH 3·0 with 1·0 N HCl and re-extracted with ether. The combined ether fractions are washed with water, dried ($MgSO_4$), filtered and evaporated to dryness *in vacuo*. The residue is dissolved in 1 ml of ethanol. Chromatographic separation is carried out on paper and on thin layers of silica gel.

Paper Chromatography

The following solvent mixture is used for the development of chromatograms: isopropanol-28% ammonia–water (80:10:10 by vol.).[18] Chromatograms were developed in darkness at room temperature (23 ± 1°).

In every test an ethanolic solution of pure IAA (Fisher, U.S.A.) ($R_f = 0{\cdot}45$–$0{\cdot}50$), the plant ethanolic extract and finally the same plant alcoholic extract plus added IAA were co-chromatographed. The Salkowski reagent was used for IAA detection.[19]

Paper chromatography was employed only for preliminary screening and, for quantitative measurements, thin-layer chromatography was used.

Thin-Layer Chromatography

Thin-layer chromatography was carried out on 250 μ layers of silica gel G[20] previously activated by heating at 110° for 40 min, using the following solvent mixture: methylacetate–isopropanol–25% ammonia (45:35:20 by vol.).[21]

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In every test an alcoholic solution of pure IAA (R_f, 0·40–0·45), the plant alcoholic extract and the plant alcoholic extract with added IAA were chromatographed.

For IAA detection the Prochazka[22] reagent was used (35% formaldehyde–25% HCl–96% ethanol, 1:1:2 by vol.). About 10 ml of this mixture were sprayed on to each 20 × 20 cm plate and the plates heated at 100° for 5 min.

In every test two identical chromatograms were simultaneously developed. One was sprayed with Prochazka reagent, and the area equivalent to the spot located on the sprayed chromatogram was removed from the second by means of the Mottier apparatus[23] which is also used directly as an elution tube. IAA elution from adsorbent was carried out with small aliquots of 95% ethanol up to 5 ml total volume. A blank was simultaneously prepared, and the IAA concentration determined at 280 mμ from a calibration curve. Recoveries of added IAA are 95%.

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