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Differential Levels of Growth Promoters in the Middle and Peripheral Grains of the Same Ear in Wheat (*Triticum aestivum* Linn. emend Thell)

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Studies conducted on the middle and two peripheral grains in four cultivars of dwarf wheat revealed that there was significant variation in grain weight at different locations. The middle grain had a significant higher weight than the two peripheral ones which when compared to each other had an insignificant variation. The various bioassays depicted that the variation in grain weight was traceable to the distribution pattern of various growth promoters. It was seen that qualitatively three different cytokinins viz., zeatin, zeatin riboside and zeatin ribotide were detectable in all the grains. However, there was a significant disparity in their quantities and the middle grains possessed more than the peripheral grains at both the stages of investigation (10 days and 35 days) after anthesis. Distribution of auxins, qualitatively three in number, was similar to that of cytokinins. There was an insignificant difference in the level of gibberellins, amounts of which declined with maturity. It is concluded that interpositional differences of growth substances was the major factor determining the difference in the grain weight and/or sink efficiency.

Key Words: Hormones, Grain Position, Growth Potential, Wheat

Introduction

Percival (1921) was perhaps the first to report that grains within the same ear of wheat, might be of different sizes and opined that this difference might be due to some physiological attribute within the grains. Since then a number of investigations have been carried out to evaluate the relative importance of diverse physiological processes and their contribution towards the grain's growth or yield. Lupton (1966) working on wheat and Hulquist and Eastin (1970) on *Sorghum* concluded that an efficient translocation system invariably contributed towards higher yield. On the other hand, Wardlaw (1968) and Bremner (1972) emphasised on the ability of individual grain to grow

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and precipitate carbohydrates rather than the translocation as the major determinant of seed growth. Asana (1974) advocated that as the growth rates of grains within the same ear and variety differed, during the early stages of grain ontogeny when the assimilates were not limiting, some inherent factor(s) must be controlling the growth of grains. Working on the hormonal control of sink efficiency in wheat, Bhardwaj and Dua (1974, 1975), Dua and Bhardwaj (1979a, 1979b) and Dua (1980a) have shown that variation among varieties with regard to 1000 grain weight was traceable to the endogenous auxin and cytokinin production of the variety vis-a-vis that of the ear. Such a postulation can further be testified by studying the levels of growth promoters in the middle and peripheral grains of wheat, which are known to differ significantly in their yield ability. With this object in view and to determine whether a relationship exists between levels of endogenous growth promoters and growth of individual grains, located at different positions, the following experiments were conducted on *Triticum aestivum* Linn. emend Thell.

Material and Methods

Four cultivars of wheat (*Triticum aestivum* Linn. emend Thell), differing in 1000 grain weight, were selected from the genetic group representing single dwarf ('HD 1553' and 'Pusa Lerma') and double dwarf ('Choti Lerma' and 'Kalyan Sona') varieties and were raised in pot culture. Sowing was done on Dec. 1, 1979 in rectangular cement pots (50 × 30 × 30 cm) containing 42 kg of soil mixed with farm yard manure. Twenty seeds per pot were sown and 20 days later seedlings were thinned to 8 and the mother shoots were tagged. The plants were grown under the optimum supply of water and nutrients. The date of anthesis was noted and the growth rate of different grains in the mother shoot was recorded from 10 days following anthesis with a regular sampling at an interval of 4 days, until maturity.

The studies on the levels of endogenous growth substances were restricted only to the cultivar 'Kalyan Sona' because of the striking and significant differences among the different grains of mother shoot at the very early stages of development. The samples for the extraction of growth regulating substances were drawn by counting clockwise the florets in the spikelet facing the upper side of flag leaf. The grain of 1st fertile floret was grouped as P_1, the next one as M followed by P_2. The grains of same age collected from three positions were grouped together and used for the estimation of different endogenous hormones. Each sample was extracted with 80 per cent ethanol at O°C in a refrigerator over a period of 24 hr. The extract was evaporated under suction to remove ethanol and the aqueous phase was utilised for the extraction of various growth regulating substances. The auxins were extracted by the method of Nitsch (1956) and gibberellins by the method of Murakami (1966). The purified extracts were chromatographed on Whatman No. 1 filter paper using iso-propanol : ammonia : water (10 : 1:1 v/v) as the solvent for developing the chromatograms. The qualitative and quantitative estimation of cytokinins was carried out by a comprehensive scheme standardised previously in this laboratory (Dua & Jandaik 1979). The ethanol extract was evaporated and the residue was mixed with water (*p*H 3). After partitioning with diethyl-ether, the aqueous extract was adjusted to *p*H 8 and extracted with n-butanol. The aqueous phase mainly contained nucleotides and was labelled as Extract I. After evaporation of n-butanol, the residue in double-glass distilled water (10 ml) was passed through a cation exchange resin (CM-Sephadex-C 50) in a 20 × 2 cm column. The column was eluted with 1 N NH_4OH

and the effluent precipitated with 1N $AgNO_3$. The supernatant was kept as Extract II, and contained most of the nucleosides. The precipitates were hydrolysed with 60 ml of 0.2 NHCl and the supernatant collected and labelled as Extract III. The three extracts were chromatographed separately on Whatman No. 1 paper, using n-butanol : acetic acid : water (12 : 3:5 v/v/v) as solvent in ascending direction. Standard series comprising zeatin, 2 iP, zeatin riboside and zeatin ribotide were run simultaneously on separate papers under identical conditions. All Rf zones, eluted with 0.3 N CH_3COOH, were tested for cytokinin activity by the *Xanthium strumarium* leaf-disc senescence technique of Osborne and McCalla (1961). Estimates of auxin activity were carried out by adopting coleoptile straight growth test as described by Mer et al. (1962) with 'Kent' oat (*Avena sativa* Linn.) coleoptiles, gibberellins by the modified technique of Ogawa (1963) using' rice (*Oryza sativa* Linn.) cultivar 'Tainan 3' seedlings. The data were analysed statistically according to the analysis of variance method.

Results

Grain Growth: The analysis (table 1) showed that all the four cultivars differed significantly in grain weight. On the whole, single gene dwarf 'HD 1553' and 'Pusa Lerma' had bolder grains than the two gene dwarfs 'Kalyan Sona' and 'Choti Lerma'. An examination of the weight of a single grain, within the same mother shoot at maturity, showed that differences due to the location of grain were very significant. The general trend was that in all the cultivars, the middle grain had higher dry matter than the two peripheral grains. In 'Kalyan Sona' the differences were significant right from the initial stages of development. At 10 days after anthesis, the middle grains of 'Kalyan Sona' had already accumulated 30.8 and 32.4% more dry matter than the P_1 and P_2 grains respectively unlike the other cultivars where significant differences were detectable only around maturity. The increment gained in 'Kalyan Sona' grains at the young stage was subsequently sustained till maturity, except at 26 days when one of the peripheral grains (P_1) picked-up to be at par with the middle grain but the growth rate declined again later on.

Distribution of Growth Promoters

(A) *Auxins*: The patterns of auxin (per unit fresh weight) in different grains (figure 1) revealed that qualitatively three auxins (Rfs 0.1–0.2, 0.6–0.7 and 0.9–1.0) were detectable. The Rf at 0.6–0.7 matched with the synthetic IAA. It was seen that all the auxins, irrespective of their chemical structure or the grains in which these were present, increased with the age of grains. The comparison of quantities (per grain) of various auxins (table 2) in different grains, varying in positions, showed significant differences at both the stages (10 days and 35 days after

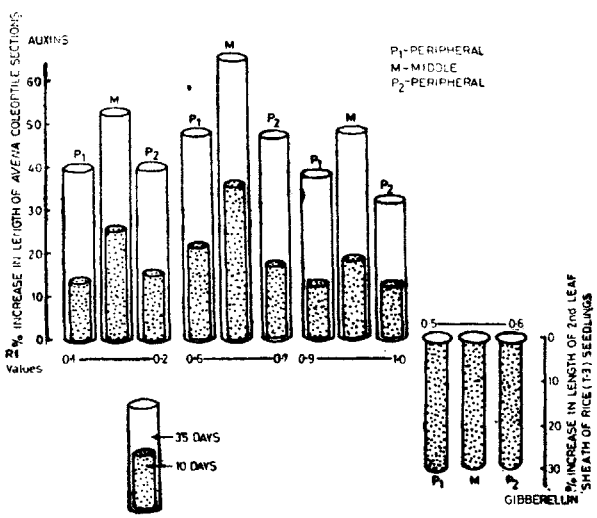

Figure 1 Auxin and Gibberellin activity in different grains of *Triticum aestivum* as revealed by different bioassays (Each sample represents 1 gm fresh weight)

Table 1 *Growth of single grain at different positions in different cultivars of wheat (Mean of 5 replications; data expressed in mg per grain of mother shoot)*

Variety	Positions	Days after anthesis								
		10	14	18	22	26	30	34	38	Maturity
HD 1553	P_1	6.56	8.89	11.60	22.82	28.26	30.13	35.13	38.12	41.25
	M	6.94	8.87	12.65	24.85	30.32	31.96	37.00	39.96	45.32
	P_2	6.84	8.83	11.76	21.60	29.12	29.16	36.10	36.47	42.35
Pusa Lerma	P_1	4.27	5.85	9.26	17.26	18.39	26.18	30.18	30.22	37.46
	M	4.93	6.07	10.26	19.35	19.71	27.71	32.00	32.64	38.42
	P_2	4.17	5.80	9.29	18.30	18.60	26.36	31.12	30.16	36.12
Choti Lerma	P_1	3.91	5.06	9.80	9.00	11.08	22.31	27.30	28.12	31.32
	M	3.95	5.08	10.00	10.00	12.97	24.33	30.33	34.30	35.03
	P_2	3.85	5.10	9.42	9.01	11.22	23.30	28.30	28.40	31.18
Kalyan Sona	P_1	3.02†	4.75†	9.00†	9.00†	12.95	21.17	28.12	28.12	31.03
	M	3.95†	5.08†	10.22†	10.33†	12.97	24.33	30.33	30.33	35.38
	P_2	2.98†	4.70†	8.97†	9.02†	11.98	21.70	28.18	28.26	32.13

Critical difference at 5% level due to position = 0.23
Variety = 0.82
Interaction = 0.42
†Significant during the early stages of development

Teble 2 *Differential levels of growth promoters in the middle and peripheral grains of the same ear (Data expressed in μg per grain basis. Average of 3 replications)*

Rf at 20°C	Age (Days after anthesis)							CD at 5% level
	10				35			
	P_1	M	P_2	Position CD at 5% level	P_1	M	P_2	
Auxins								
0.1–0.2	1.82	2.13	1.83	0.13	2.46	3.12	2.46	0.21
0.6–0.7	2.03	2.34	1.94	0.16	2.81	3.52	2.81	0.16
0.9–1.0	1.81	1.91	1.75	0.12	2.69	2.82	2.69	0.18
Total	5.66	6.38	5.52	0.17	7.96	9.46	7.96	0.36
Gibberellins								
0.4–0.5	0.139	0.120	0.116	0.024	Tr	Tr	Tr	—
0.7–0.8	0.204	0.026	0.023	0.013	Tr	Tr	Tr	—
Total	0.343	0.146	0.139	0.027	—	—	—	—
Cytokinins								
0.1–0.2	0.021	0.048	0.020	0.013	0.049	0.071	0.051	0.010
0.2–0.3	0.006	0.012	0.006	0.004	0.031	0 051	0.038	0.013
0.4–0.5	0.008	0.049	0.008	0.011	0.050	0.071	0.052	0.015
Total	0.035	0.109	0.034	0.016	0.130	0.193	0.141	0.062

Tr Traces

anthesis). The middle grain had unequivocally higher amount of total auxins (11 and 17% more than P_1 and P_2 grains, respectively). The total as well as individual auxins increased in all the grains with age but even here the accumulation of auxin per unit time was faster in the middle grain and consequently the difference in auxin, between the middle and peripheral grains, further widened. The quantity of auxins in the two peripheral grains at the two stages of development did not differ significantly. The individual auxins were more or less similar in behaviour to those of total auxins.

(B) *Gibberellins:* The distribution of gibberellins showed that the most conspicuous extractable gibberellin from the grain was at the Rf (0.4–0.5) matching with the synthetic GA_3 (figure 1). Another gibberellin (Rf 0.7–0.8) was also detectable in traces and its equivalents are given in table 2 but this contributed very little to the total content of gibberellins (14.8% of the total gibberellins). Gibberellins declined with age; the decline was so rapid that it was hardly detectable at 35 days after anthesis. Interpositional comparison of gibberellins showed that there was insignificant difference in their levels among the three grains.

(C) *Cytokinins*: Three cytokinins matching with synthetic zeatin riboside (Rf 0.1–0.2), zeatin ribotide (Rf 0.2–0.3) and zeatin (Rf 0.4–0.5) could be isolated while at Rf 0.8–0.9 and 0.2–0.3 which corresponded with adenine and 2iP respectively, no activity was detectable at both the stages of development (figure 2 and Table 2). The level of all cytokinins increased with age in the grains irrespective of their position. The quantitative difference at 10 days was apparent and the central grain had 10.9 and 13.7 per cent higher total cytokinins than the P_1 and P_2 grains respectively. The distribution pattern was similar in 35 day grains i.e., the middle grains were with higher cytokinin levels. The quantity in the middle grains increased at a faster rate than in the peripheral grains. The behaviour of individual cytokinins was similar to that of total cytokinins and in quantity zeatin was maximum followed by zeatin riboside and zeatin ribotide.

Discussion

The potentiality of a grain to grow and accumulate photosynthetic assimilates has been recognized as an important parameter determining grain yield in cereals. Percival

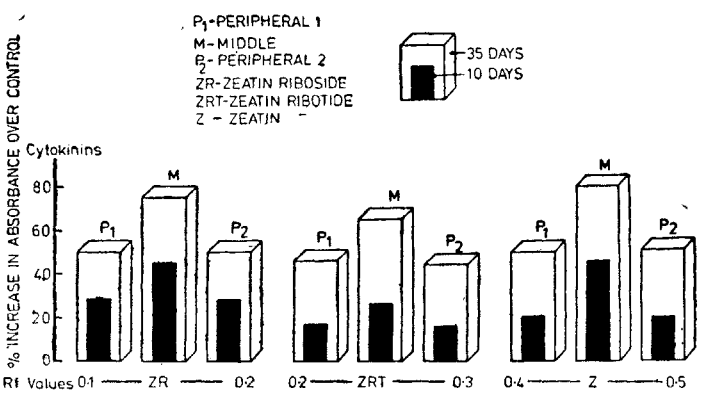

Figure 2 Cytokinin activity in different grains of *Triticum aestivum* as revealed by *Xanthium* leaf disc bioassay (Each sample represents 1 g fresh weight)

(1921) suggested that the difference in grain size was attributable to either (i) longer period for starch deposition in some grains or (ii) competition for assimilate among grains or (iii) hormonal interaction amongst them. Kiesselbach (1948) added another component to the list namely higher translocation rate. Besides these parameters, a number of reports have also favoured other miscellaneous factors like photosynthesis (Birecka & Wlodkoswska 1966), leaf area (Watson 1952) or leaf area duration, (Wellbank et al. 1966) which singly or jointly imparted a higher yielding ability to a cultivar. However researches conducted in the last three decades (Asana & Williams 1965, Asana et al. 1969, Bingham 1969, Rawson & Ruwali 1972) showed that the primary determinant of yield in wheat was sink efficiency (build up by grain size and number). Evans et al. (1972) mooted that the interaction of growth regulating substances with the sink efficiency might be involved in influencing the yield potential in wheat. Subsequent work done on this line has shown (See under Dua & Bhardwaj 1974–79, Dua 1980a) that differences in varietal yield were traceable to the differences in the endogenous growth substances viz., auxins, gibberellins and cytokinins. A correlation between grain size and auxins and cytokinins on the one hand and gibberellin and grain number on the other was established. The present findings show that difference in respect of the weight of individual grains could also be ascribed to the difference in the level of phytohormones. Thus, the middle grains, which were endowed with better capacity to grow had a higher level of individual as well as total auxins and cytokinins at both the stages (10 and 35 days) while no specific role could be assigned to gibberellins in relation to grain growth. The role of gibberellin in grain development and possible causes of its reduction are discussed elsewhere (Dua & Bhardwaj 1979b). The present studies further show that the sink efficiency is probably a function of a balance of diverse endogenous growth substances as Dua (1980b) has reported that abscisin like substances, present in the grains of *T. aestivum*, at the initial stages of grain development, varied with the position of the grain and that the peripheral grains possessed more inhibitors than the middle grains although at all the positions these declined with age. The presence of higher endogenous hormones in the middle grain might be responsible for higher dry matter precipitation via enhancement of mobilisation of translocate (Mullins 1970) and/or an improvement in the storage capacity of the grains as advocated by Humpries (1963) and Sweet and Wareing (1965).

To conclude, it is suggested that the positional differences, in respect of grain yield are based on the differences in the efficiency and capacity of individual grain to grow which in turn appear to be related to the endogenous level or balance of auxins, cytokinins and probably inhibitors.

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