Relative Levels of Methionine, Tryptophan and Proline as Affected by Salicylhydroxamic Acid Within Developing Grains of Wheat

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ABSTRACT

The salicylhydroxamic acid (SHAM) effects on relative levels of methionine, tryptophan and proline were studied within developing grains of wheat (Triticum aestivum L. var. PBW-343). The plants were grown in a screen covered hall under otherwise natural conditions. A concentration of 10 ppm salicylhydroxamic acid was applied at anthesis stage in five replications with the help of cotton plugs, which remained on ears of mother shoots for 48 hours. Mother shoots were sampled five times, seven-day intervals started from seventh day after anthesis (DAA) up to 28th DAA, and at maturity. The spikelets were divided into two grain types included basal (bold) and apical (small). The salient points emerging through the use of salicylhydroxamic acid were that (i) both bold and small grains showed a significant increase in relative levels of methionine and tryptophan from 14th DAA stage, while the relative levels of proline decreased, and (ii) in spite of aforementioned changes, they continued to exhibit the disparity between them and at maturity the smaller grains still showed lower methionine, tryptophan and proline than the bolder grains.

Key words: Amino Acids; SHAM; CN-resistant respiration; Triticum aestivum L.

Introduction

Since the dawn of civilization the grain of food gave life and kept hunger and death at bay. It became the staple around which homes and communities evolved. Despite the impressive advances made in improving its production, over the centuries there is little reason to become complacent about its supply, especially in the developing world. A casual look into the present global food supply reveals that the cereals constitute 2/3 component of its resource. An appraisal of parameters regulating their productivity divagles that their full potential to yield is still unrealized. One of the grey areas, which have remained untapped, is the host of physiological and genetical barriers of developing kernels to grow to an optima and their manipulation by desirable traits and methodologies. The potential up gradation of components constituting the total yield in wheat (number of productive tillers m⁻², grains per spike and 1000-grain weight), would help to raise the production substantially. Though, significant milestones have been achieved in the first two parameters the last component, the individual grain weight has eluded scientific investigations and rather paradoxically has declined with the advent of high yielding varieties. A study into the physiology of grain yield shows the existence of variation among different varieties or genotypes or even the grains developing in the same ear [3,26,22,30,11]. It further discloses that the yield may be influenced by the availability of photosynthates to the developing sinks [31,23,24,9]. Various sugar responsive genes in plants potentially affect the partitioning (Geiger et al. 1996) and have been stressed to be key determinant of plant productivity [10]. Dry matter partitioning also
plays a paramount role in growth rate of sink organs (Heuvelink and Bertin 1994). Working on the grain growth in wheat and buckwheat variation among varieties was traceable to endogenous hormone production in variety vis-à-vis that in the ear [8,7]. A few biochemical components as advocated by Abrol et al. [2], Hakaka [12] and Hasan and Kamal [13], might be of significance in determining sink efficiency and/or the grain yield. Since, the harvest index is the culmination of innumerable events, most of the view points on sink efficiency appears to be speculative and need a holistic approach in isolating obligatory events to produce the net assimilates. The revelation that the electron transport chain, in operation during biological oxidation, might find an alternate route without performing the target aim of creating proticity and may downgrade the overall impetus of meristemts to grow by 10 to 25 percent [25]. Indeed, it has been reported that higher alternative respiration could be one of the reasons of lower growth of grains at distal position in a spikelet [27]. It is, therefore, advocated that any attempt to interrupt this process may prove beneficial in improving productivity. In the present study, it is proposed to analyze the relative levels of few amino acids namely methionine, tryptophan and proline as affected by specific inhibitor of salicylhydroxamic acid (SHAM) in different grains growing in the same spikelet of wheat.

**Materials and methods**

The investigation was conducted with a common bread wheat (Triticum aestivum L. var. PBW-343), which was sown in circular earthenware pots (50x30x30 cm) containing 35 kg of soil mixed with farmyard manure (4:1). Eight seeds per pot were sown and after 15 days, seedlings were thinned to two. Hoagland's nutrient solution [16] was supplied to the pots. The plants were grown in a screen covered hall under otherwise natural conditions. A concentration of 10 ppm salicylhydroxamic acid was applied at anthesis stage in five replications with the help of cotton plugs, which remained on ears of mother shoots (MS) for 48 hours. The labeled main spikes were sampled five times, seven-day intervals started from seventh day after anthesis (DAA) up to 28th DAA, and at maturity. Grains were usually taken from three different segments in the ear. The labeled samples of grains were brought to laboratory and separated to two types of grains (small and bold) and the following amino acids analysis were carried out in the above aged grains.

The methionine, tryptophan and proline content were determined by methods described by Horn et al. [17], Mertz et al. [20], and Batez et al. [4], respectively.

**Results and discussion**

The salient points emerging through the use of salicylhydroxamic acid were that (i) both bold and small grains showed a significant increase in relative levels of methionine and tryptophan from 14th DAA stage, while the relative levels of proline decreased (Figures 1, 2 and 3), and (ii) in spite of aforementioned changes, they continued to exhibit the disparity between them and at maturity the smaller grains still showed lower methionine, tryptophan and proline than the bolder grains (Figure 4).

The representation of the data in Figure 1 indicates the level of methionine as influenced by salicylhydroxamic acid in different grains growing in the same spikelet of wheat. With regard to the levels of tryptophan a similar pattern as that of methionine was recordable. The relative levels of this amino acid increased rapidly in first 14 days post-anthesis stages followed by a gradual decrease which continued until maturity. The fall was to the tune of 38.7 percent at 21st DAA which further fell by another 33.1 percent at 28th DAA followed by another decline of 23.4 percent at maturity, thereby showing an overall declension to the tune of 68.6 percent in bold grains. A more or less similar pattern was apparent in smaller grains, which despite the fact being endowed with lower levels of methionine showed a declension right from 14th DAA onwards, reaching to a minimum level at maturity. The distribution of the enzyme was unequivocally lower in smaller grains than bolder grains at all the stages of investigations e.g., at 7th DAA its level was lower by 19.7 percent and by 25.1, 21.1 and 16.4 percent more at 28th DAA stages respectively with a final figure of 12.8 percent less in smaller grains at harvest (Figure 4).

A comparative look into the levels of tryptophan and its distribution in bold and small grains, disclosed that smaller grains were endowed with its relatively lower levels at all stages of investigations. The analysis of data revealed that the lower quantum of distribution in smaller grains was maximum at 14th DAA (28.7 percent less than bolder grains) and subsequent to that the differences were to the tune of 19.8, 18.2 and 14.6 percent less in smaller grains at 21st and 28th DAA and at maturity (Figure 4). At 7th DAA smaller grains possessed relatively 23.2 percent
lower tryptophan than their counterpart bolder grains. As apparent from the data in Figure 3, the relative levels of proline increased in first 14 days post-anthesis stages followed by a gradual decrease which continued until maturity. The fall was to the tune of 29.4 percent at 21st DAA which further fell by another 31.8 percent at 28th DAA followed by another decline of 36.5 percent at maturity, thereby showing an overall declension to the tune of 69.2 percent in bold grains. A more or less similar pattern was apparent in smaller grains, which despite the fact being endowed with lower levels of proline showed a declension right from 14th DAA onwards, reaching to a minimum level at maturity.

The analysis of data revealed that the higher quantum of distribution in bold grains was highest at 21st DAA (17.8 percent higher) and subsequent to that the difference was to the tune of 16.8 and 16.0 percents more in bold grains at 28th DAA and at maturity. At 7th and 14th DAA the smaller grains possessed relatively less (15.2 and 11.1 percent respectively) of proline than their co-developer bolder grains (Figure 4).

The results bring forth, in no uncertain terms, the findings that the ear of wheat is a developing place for a definite number of grains which intern are separate biological entities endowed with their inherent potentials. This axiom was advocated by Abolina [1] and is in line with the observations of innumerable workers [6,19,28,30]. Nevertheless, the sequence of events, piloting the yielding ability, is the metabolic profile and if augmented through the use of plant growth regulators [29,18] or by imposing a shift in metabolic events [7] promotery effects are achievable [14,21]. In present context, the central point which came to light in the present endeavor is that an unusual path of aerobic respiratory chain (CN-resistant respiration) plausibly switches-on during the grain filling stage and if checked, through the immaculate use of salicylhydroxamic acid, can significantly increase the relative levels of methionine and tryptophan in the grains. Of course, SHAM or regulator of alternate oxidase pathway was not successful in eliminating the disparities between the two types of grains.

![Fig. 1: Relative levels of methionine (percent in grain) at different location within developing grains of wheat (Triticum aestivum L. var. PBW-343) as influenced by salicylhydroxamic acid; Values within parenthesis indicate percentage of increase (+) in level of methionine over control](image1)

![Fig. 2: Relative levels of tryptophan (percent in grain) at different location within developing grains of wheat (Triticum aestivum L. var. PBW-343) as influenced by salicylhydroxamic acid; Values within parenthesis indicate percentage of increase (+) in level of tryptophan over control](image2)
Fig. 3: Relative levels of proline (µmol g\(^{-1}\) fresh weight) at different location within developing grains of wheat (*Triticum aestivum* L. var. *PBW-343*) as influenced by salicylhydroxamic acid; Values within parenthesis indicate percentage of increase (+) or decrease (-) in level of proline over control.

Fig. 4: Percentage of decrease (-) in relative levels of methionine, tryptophan and proline in small grains over their counterparts bold grains.

References