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The Role of the Flag Leaf in the Formation of the Yield of Winter Wheat (*Triticum aestivum* L.) Plants

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Abstract

During the period of heading and grain ripening, the characteristics of photosynthesis of the flag leaf and the structure of the yield of wheat plants of the varieties Moldova 5, Missia, and Kuyalnik were studied. The plants were cultivated in 2016-2019 in the experimental field of the Institute of Genetics, Physiology, and Plant Protection of the Academy of Sciences of Moldova, on the outskirts of Chisinau. It was shown that the total duration of the phases of grain formation and ripening in an ear of wheat variety Moldova 5 was 26 days, and for the Misiya and Kuyalnik varieties, it was 33 days. At all phases of grain formation in the Moldova 5 e variety, there was a tendency towards the manifestation of lower photosynthetic activity of the flag leaf compared to that detected in the flag leaf of the other two wheat varieties. During three years of cultivation, the estimated wheat grain yield in Moldova 5, Missia, and Kuyalnik varied from 3.76 to 4,20 - 3,82 - 4,30 and 3,80 - 4,25 tons per hectare. The obtained data suggest that based on a comparison of the specifics of the daily dynamics of PS2 activity in the flag leaf of plants being in the same phase of grain development, it is possible to distribute wheat genotypes according to their productivity under given environmental conditions. Because the duration of grain formation and ripening in the ear varies depending on environmental conditions (mainly temperature and humidity), to correctly determine the potential productivity of the genotype, testing of the wheat varieties should be carried out in different climatic zones. **Keywords:** Hexaploidy Wheat; Varieties; Flag Leaf; Photosystem Two; Heading Phases; Productivity

Introduction

Wheat is one of the most important plants [46]. Around the world, about 2.1x10⁸ hectares are sown with this crop, resulting in approximately 6x10⁸ tons per head [47]. Although over the past 40 years, the sown area has not increased by more than 10%, thanks to the introduction of modern agricultural technology and improved varieties, wheat yields have tripled [48]. Despite this, the demand for wheat consumption is constantly growing. This is evidenced by the price of wheat, which has doubled over the past decade [49]. At the same time, adverse climate change is occurring, with average temperatures rising and water availability decreasing in many wheat-growing areas [50]. This is especially typical for the Republic of Moldova, located in a zone of risky agriculture [24,36]. Thus, the rational use of existing varieties and the selection of new wheat genotypes resistant to extreme temperatures and drought is an urgent task.

The growth, development, and productivity of wheat plants are complexly dependent on the intensity of photosynthesis [22,34]. The complexity of this dependence is especially pronounced in higher plants, in which leaves of different ages and locations specifically influence the growth, flowering, seed formation, and the accumulation of reserve substances in them. In general, the harmonious growth and development of plants depends on the specific integration of the effects of photosynthetic activity of leaves of different ages and the level of their location on the plant. To study these features, it is convenient to use wheat plants, in which new leaves are formed strictly in the acropetally direction. In them, the formation of new leaves stops after the initiation of heading, which is accompanied by flowering, formation, and ripening of seeds, aging, and death of the plant. Integrative, the occurrence of these processes depends on the photosynthetic activity of all leaves, but the relative role of leaves of different levels on the efficiency of each of

these processes is specific. It should also be noted that the assimilating organs of winter wheat are not only the leaves, but also the stem, ear, leaf sheaths, and awns [10,21]. As with leaves of different levels (ages), the importance of photosynthesis processes in each of these organs in determining the yield depends on the stage of plant development [15,37,41].

The productivity of wheat plants is assessed by the accumulation of total biomass (mainly in the aboveground part) and grain biomass [2,33]. Over the years, thanks to advances in breeding, the yield of wheat varieties has increased, as well as its rate from the total aboveground plant biomass. In modern wheat varieties, it reaches 0.55 [41]. According to the results of researchers [7,9,31], up to 90 - 95% of the dry biomass of grains is created due to the process of photosynthesis, which takes place in all plant leaves, and only 5 - 10% of their biomass is formed due to photosynthesis in the stem, ear, leaf sheath, and awns. It is for this reason that, as a rule, the size of the assimilating surface of plants is often characterized only by the leaf surface area. Works devoted to studying the influence of the leaf area on yield, productivity, and its elements evidence this [8,19,26]. Because of these studies, a relationship was established between plant yield and leaf surface area. As a result, the authors recommend creating such crops and agrotechnical conditions for winter wheat plants in which the leaf surface index reaches 40 - 60 thousand/ha [14,32]. At the same time, there is information (S.F. Lyfenko) [28] that a large leaf area, because of an increase in the total intensity of water evaporation by stomata, can lead to a decrease in drought and heat resistance of plants. In addition, according to V.V. Maimistov [29], during the grain-filling period, drought-resistant genotypes have increased, but not the highest, foliage indicators [23,39]. During this period, only the upper leaves have high assimilating activity, the size of which closely correlates with the productivity of the ear. The correlation coefficient between leaf area and such indicators as grain weight in an ear, number of grains in an ear, and weight of 1000 grains is +0.65 - 0.97: +0.48 - 0.97 and +0.22 - 0.96 respectively [25,44,45]. In this regard, the author concluded that it is necessary to select varieties with a long period of maintaining the viability of these particular leaves. According to the works of V.I. Lukyanyuk [27], daily increases in dry matter in the period after the initiation of the heading phase are largely determined by the productivity of photosynthesis per unit of the leaf surface. At the same time, according to [6,11,16], an increase in leaf area does not always have a positive effect on the yield of cereals. According to their data, photosynthesis of cereal leaves plays a leading role in determining plant productivity only in the first half of the growing season, while starting from the heading phase, photosynthesis in organs such as

the ear and stem becomes increasingly important [5,37]. According to some researchers, the share of ear photosynthesis in the supply of grains with plastic substances is 10 - 40% [17], 50 - 60% [1,38], or even 90% [40].

The above data indicate that there is still no consensus on the role of various organs in determining the productivity of cereal crops. At the same time, there is no doubt that leaf photosynthesis plays the principal role in providing wheat plants with plastic substances at different stages of grain formation and ripening in the ear. From a practical point of view, it is of particular interest to identify the specific role of photosynthesis activity in the flag leaf of wheat, which appears last and remains highly photosynthetic active during grain formation and filling in the ear. Based on the mentioned, our research goal was to identify possible correlations between the dynamics of photosynthesis activity in the flag leaf of different wheat varieties during the grain formation and ripening periods and the genotype yield.

Materials and Methods

We carried out the research over three years, from 2015 to 2018, of wheat cultivation at the experimental field of the Institute of Genetics, Physiology, and Protection of Plants of the Academy of Sciences of the Republic of Moldova. The research objects were three winter wheat varieties: Moldova 5, Missia, and Kuyalnik. The agricultural technology for cultivating winter wheat met generally accepted requirements for this climatic zone [30,35,41]. The predecessor was black steam. The seeding rate was 5.5 million seeds per hectare.

At the end of the heading stage, when the leaf sheaths were bent and leaf growth stopped, in each of the three replications, the area of the flag leaf of the central stem of ten plants was determined by the weight method. The intensity of flag leaf photosynthesis at various stages of grain formation and ripening (flowering, grain growth, milky ripeness, and milky-waxy ripeness) was assessed based on the activity of photosystem two (PS-II), using a PAM-2100 fluorimeter (WALZ, Germany) Yield indicator of the flag leaf for ten central (main) shoots, in triplicate. Mathematical processing of the data was carried out using the Microsoft Excel 2007 program according to the field experience methods, described in the monograph [3,18], calculating the average indicators, dispersion, and reliability of the difference between the averages of different options.

Results and Discussion

In our studies, we have used winter wheat varieties Moldova 5, Missia, and Kuyalnik. Data on the morphological parameters of the flag leaf of plants of the indicated wheat varieties grown in 2016, 2017, and 2018 are given in Table 1. The obtained results suggest that the studied wheat varieties, regardless of the year of cultivation, in terms of leaf area and length, can be located in the following order: Moldova 5 > Missia > Kuyalnik. At the same time, for the

Missia variety, the relative width of the flag leaf in plants grown in 2016 and 2017 was higher than in the other two wheat varieties, and in those obtained in 2018, it was lower. Despite the lower width in plants of the variety Missia, in 2018, due to the greater length of the flag leaf, its area remained higher compared to that of plants of the Kuyalnik variety.

Varieties	2015-2016			2	2016-2017		2017-2018			
	Leaf area	Length	Width	Leaf area	Length	Width	Leaf area	Length	Width	
Moldova 5	18,9	21,8	1,14	20,2	28,3	1,18	21,8	29,0	1,21	
avr.	±1.9	±1.5	±0.09	±1.63	±1.23	±0.08	±1.24	±1.28	±0.03	
Missia	15,8	18,4	1,16	19,3	23,3	1,27	19,5	24,3	1,15	
avr.	±1.82	±1.1	±0.07	±1.45	±1.0	±0.03	±1.51	±0.9	±0.02	
Kuyalnik	14,7	17,2	1,12	18,9	20,0	1,14	19,1	20,5	1,17	
avr.	±1.36	±1.6	±0.06	±1.24	±0.9	±0.02	±1.28	±0.9	±0.03	

Table 1: Parameters characterizing the size of the flag leaf area of winter wheat varieties Moldova 5, Missia, and Kuyalnik.

To characterize the photosynthetic activity of the flag leaf of plants of the studied wheat varieties, which are at different stages of seed maturation in the ear, the dynamics of changes in the quantum yield (Yield) of photosystem II were determined based on the fluorescence of leaf chlorophyll, measured using a PAM 2100 fluorimeter (Germany). Fluorescence was determined throughout the entire photoperiod at different intensities of daylight. This method makes it possible to evaluate the flag leaf daily dynamics of the photosynthetic activity of plants that are at specific stages of grain formation and ripening. More than that, the method made it possible to avoid leaf damage when determining photosynthetic activity. Thanks to this, we were able to measure the photosynthetic activity of the flag leaf of the same selected plants that were at different stages of grain formation and maturation in the ear. The obtained data are shown in the figure. The figure 1 shows that with increasing illumination in the morning, the values of the Yield indicator consistently fell from the maximum level detected at 8 a.m., PAR 860 µmol*m-2*s-1, until the PAR value reached 1100 µmol*m-2 *s-1, at noon. Then, as the PAR level increased to 1400 µmol*m-2*s-1, at 13:00, the Yield values dropped sharply to 100 and below, remaining at a low level until 15:00, after which the Yield value increased with a drop in the PAR level below 1000 µmol*m-2*s-1. It is important to note that as the grain matured in the ear, the maximum value of the Yield index consistently decreased from 0.420, for the flag leaf in the heading initiation phase, to 210, for the flag leaf of plants that were in the milky-wax ripeness phase. In the evening, regardless of the wheat variety, with a decrease in PAR levels, the increase in Yield as the leaf aged slowed down. As noted above, the aging of the flag leaf and ripening of grain in the

ear were accompanied by a gradual decrease in Yield values determined at 8 a.m. As the grain ripened, regardless of the wheat variety, the difference between the maximum Yield value of the flag leaf achieved at 7:00 p.m. and that which was detected at 8:00 in the morning consistently changed from positive, in the flowering and grain growth phases, to negative, in the milk and grain growth phases' milky-waxy ripeness. These data indicate that as the flag leaf ages, the dynamics of photochemical restoration of inhibited centers decrease faster than the dynamics of their inhibition in the morning. Because of this, the difference between the Yield level of the flag leaf at 7:00 p.m. and 8:00 in the morning gradually changed from positive to negative values as the grain ripened.

Quantitatively, in the studied wheat varieties, the daily dynamics of changes in Yield values as the grain ripened in the ear differed significantly. In the morning, the fall in the Yield indicator of the flag leaf of the Moldova 5 variety plants, which were in the flowering phase, passed faster. In the evening, it recovered more slowly than in the flag leaf of the other two varieties. These differences persisted after 12 hours only in plants that were in the phase of grain growth and milk ripeness. They practically disappeared in plants that entered the stage of milky-wax ripeness. In general, the data presented in the figure indicate that in the morning, with increasing illumination, in plants of the Moldova 5 variety, the inhibition of photosynthetic activity of the flag leaf was more intense compared to that which was characteristic of the flag leaf of plants of the Missia and Kuyalnik varieties. In the afternoon, as PAR values decreased, the following trend was revealed in the Yield dynamics: the rate and level of restoration of photosynthetic activity of the flag leaf decreased as the grain ripened in the ear.

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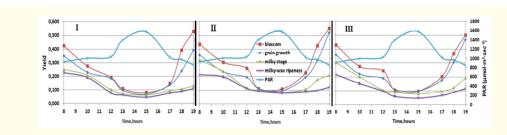


Figure: 1 The activity of FS -2 of the flag leaf of winter soft wheat varieties I – Moldova 5, II- Mission, III- Kuyalnik in various phases of seed maturation.

It is important to note that the Yield values of the flag leaf decreased phases of flowering, grain growth, milky ripeness, and milky-wax ripeness of plants, respectively, reaching 430, 350, 310, and 210. These decreases in the values of the Yield indicator were due to the consistent aging of the flag leaf and the whole plant.

When assessing the biological characteristics of wheat varieties, it should be borne in mind that plant productivity may depend not only on the rate of the flag leaf aging but also on the duration of the phases of grain formation and ripening in the ear. According to our data, the flowering, grain growth, and milky and milkywax ripeness phases duration of the Missia and Kuyalnik varieties plants cultivated in 2018 was the same, and respectively lasted 7, 12, 8, and 6 days, while in the Moldova 5 variety, they were respectively lasted 6, 10, 6 and 4 days. These data indicate that in comparison with plants of the Missia and Kuyalnik varieties, plants of the Moldova 5 variety were observed not only more rapid flag leaf aging but also a more rapid passage of the phases of grain formation and ripening in the ear. The total duration of these phases of the Moldova 5 variety plants was 26 days, and in the plants of the other two varieties - 33 days. Thus, if we take into account that the degree of tillering in these varieties of wheat plants obtained after sowing 5.5 million seeds per hectare was almost the same (equal to 1.15), we assume that the photosynthetic activity of the flag leaf significantly influenced the formation of the yield of wheat plants, expected yield of the Missia and Kuyalnik varieties may exceed the plants of the Moldova 5 variety.

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Data on the structure and yield level of the wheat varieties Moldova 5, Missia, and Kuyalnik are shown in Table 2. They indicate that the variety Moldova 5, despite the large size of the flag leaf, has all productivity indicators, including estimated productivity per 1 hectare showed a general tendency to be lower compared to the corresponding values characteristics for the Missia and Kuyalnik varieties. Firstly, attention is drawn to the fact that despite the same sowing density, the number of plants per square meter for the Moldova 5 variety in different years of cultivation was lower than the corresponding value for the other two varieties. It follows that seed germination and survival of plants of this variety were lower, regardless of the year of cultivation. Additionally, the productivity of plants of the Moldova 5 variety was negatively affected by the comparatively lower number of grains produced in the ear of the central plant. In general, these factors led to the fact that the estimated yield per hectare of plants of the Moldova 5 variety was 40 - 100 kilograms less than what was typical for the Missia and Kuyalnik varieties.

Parameters	2015 - 2016			2016 - 2017			2017 - 2018		
Parameters	Moldova 5	Missia	Kuyalnik	Moldova 5	Missia	Kuyalnik	Moldova 5	Missia	Kuyalnik
Yield, t/ha	3,76	3,82	3,8	3,93	3,99	3,96	4,2	4,3	4,25
Length of a large ear, cm	7,8	7,6	7,7	7,8	7,7	7,5	7,6	7,7	7,7
Number of medium ears, pcs/m2	115	118	116	136	140	142	124	132	133
Number of ears, pcs/m2	585	535	531	554	528	528	514	520	512
Number of small ears, pcs/m2	470	417	415	418	388	386	390	388	379
Number of grains from an average ear, pcs	25,4	28,6	28,8	26,1	28,4	27,9	29,3	29,9	30,1
Number of grains from a small ear, pcs	18,2	18,6	18,7	19	18,9	18,8	19,7	19,5	19,6
Grain weight per ear, g	1,15	1,23	1,21	1,21	1,25	1,24	1,21	1,26	1,27
Weight of 1000 grains, g	32,2	34,4	34,2	34,2	35,3	35,6	36,7	37,4	37,2

Table 2: Variation in the structure of harvest and the yield of winter wheat plants, varieties Moldova 5, Missia, andKuyalnik, cultivated in 2016-2019.

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The data presented in Table 2 differ from the results of studies by other authors [20,43], who showed that with an increase in the width of the flag leaf of wheat plants, the size of the spike of the central plant, the total number of productive spikelets, the grain content and productivity of the spike increase, the height of plants and the number of unproductive spikelet's decreases. The results of our studies demonstrated that the relative width of the flag leaf of the studied wheat varieties can vary, and increasing its width is not accompanied by an increase in plants' relative yield. For example, in 2019, plants of the Moldova 5 variety had the largest flag leaf width (Table 1) and, at the same time, the lowest productivity (Table 2). The unpredictable and different direction of variation in the relative width of the flag leaf from year to year in different wheat genotypes gives reason to believe that its width cannot serve as a reliable indicator of highly productive wheat varieties used for breeding.

According to our data, the duration of the phases of flowering, grain growth, and milky and milky-wax ripeness in plants of the Missia and Kuyalnik varieties cultivated in 2018 was the same and, respectively equaled 7, 12, 8, and 6 days, while in the Moldova 5 variety, they were respectively equaled 6, 10, 6, and 4 days. These data indicate that plants of the Moldova 5 variety, in comparison with plants of the Missia and Kuyalnik varieties, observed not only more rapid aging of the flag leaf but also a more rapid passage in the ear of the phases of grain formation and ripening. The total duration of these phases in the Moldova 5 variety plants was 26 days, and in the other two varieties - 33 days.

The yield of wheat plants depends significantly on weather conditions during the period of heading and grain ripening. During this period, the biochemical processes in the ear influence the number of grains and the number of cells in the endosperm of each grain depends on the number of cells in the endosperm and the level of accumulation of reserve substances in each cell to determine the total dry matter content in the grain [13]. Intensive formation of cells in the endosperm occurs during the flowering and grain growth phase. Then, in the milky and milky-waxy phase of ripening, the cells are filled with reserve substances [6,13]. Thus, the yield of wheat plants also depends on the fulfillment of processes characteristic of all four phases of grain formation. Our data confirm these conclusions. The total period from grain formation to its ripening for the Moldova 5 wheat variety was 26 days and for the Missia and Kuyalnik varieties - 33 days. As a result, the accounting grain yield per hectare for wheat plants of the Moldova 5 variety was 3.76, and for plants of the variety Missia and Kuyalnik-3.8 t/ha (Table 2). The differences noted above in the yield of wheat varieties Moldova 5 and the Missia and Kuyalnik varieties may depend on differences in the dynamics of photosynthesis intensity during the day. In the wheat variety, Moldova 5, the intensity of photosynthesis in all phases of grain formation and ripening tended to be

lower than in the other two varieties, see figure. It is also important to emphasize that the quantity and quality of the wheat harvest significantly depends on the specifics of the processes taking place in all phases of the formation and ripening of grain in the ear. The influence of high temperatures and drought on the formation and division of endosperm cells has been studied less fully compared to their influence on the processes occurring in the phases of accumulation of reserve substances and grain ripening [6]. It has been shown that under conditions of heat and drought, processes such as grain ripening in the ear, inhibition of starch biosynthesis, cell apoptosis in endosperm tissues, and the achievement of physiological grain maturity are accelerated [4]. Under the influence of high temperatures during the grain-filling period, the expression of gluten biosynthesis genes accelerated in the wheat ear. As a result of these changes, an acceleration of grain ripening was observed, which led to a decrease in the period from the beginning of heading to the ripening of grain in the ear [4,16]. Probably, these phenomena occur since drought and heat accelerate the processes taking place in the embryo and endosperm, due to which grain ripening is accelerated.

In connection with the results noted in this work, we cannot exclude the possibility that the lower estimated productivity of plants of the variety Moldova 5 compared with that of the o varieties Missia and Kuialnik, Table 2, is not associated only with lower potential productivity. Plant productivity is influenced by environmental conditions, which for the Moldova 5 variety turned out to be more severe than for plants of the Missia and Kuyalnik varieties. So, the conclusion about the differences in stress resistance and productivity of these wheat varieties can be made only after analyzing the results of their cultivation in different climatic zones.

Conclusions

- Wheat plants, in which the daily activity of photosynthesis of the flag leaf is higher during the passage of various phases of grain formation and ripening in the ear, tend to be characterized by a more extended period of passing through each of grain ripening phase in an ear, starting from the initiation of heading and ending with the grain ripening phase.
- Based on the specifics of the daily dynamics of PSII activity in the flag leaf of plants passing through the same grain development in the ear phase, it is possible to distribute wheat genotypes according to their productivity under given environmental conditions.
- Since the duration of grain formation and ripening grains in the wheat ear varies depending on the environmental conditions (mainly temperature and humidity), testing the productivity of wheat varieties should be carried out in different climatic zones to determine the potential productivity of the genotype.

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