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REVIEW: SOME PHYSIOLOGICAL INDICES TO BE EXPLOITED AS A CRUCIAL TOOL IN PLANT BREEDING

ABSTRACT

This article is mainly addressed to plant physiologists and breeders. Nowadays, the cooperation between these two groups seems to be more important than ever before. Plant physiology offers better understanding of mechanisms and factors responsible for plant yielding. Thus, it might help to find proper traits for plant selection. Plant breeding proposes highly differentiated material for testing.

Key words: chlorophyll fluorescence, gas exchange, growth analysis, plant breeding, physiologica l indices, salt stress, selection criteria, yielding.

INTRODUCTION

A substantial progress in biology has been recently achieved thus, assuring the development of modern agriculture (Nalborczyk 1998). This is illustrated in Table 1, which shows the impact of the principal factors affecting crop productivity. The use of fertilization was the main factor affecting crop productivity during the years 1951-1970 while the use of biological progress has been responsible for productivity increase through the years 1971-1998. Nevertheless, better use of available modern methods, techniques and cooperation between different agricultural disciplines might accelerate this progress.

The plant breeder has -for a long time- been the one who works under field conditions using skilled hands and experienced eyes to obtain mainly one goal - the achievement of higher final yield, while its quality being treated as a secondary one. In addition, he has been always using a large scale selection and crossing methods what means, that he has not enough time for analysis of the necessary biochemical and physiological parameters. Looking for the characteristics of crop ideotypes he narrowed his interest only to the ones directly related to yield like num-

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ber of ears per plant, number of grains per ear, thousand corn weights etc. Such approach leads to a loss in the crops' quality and narrowed crop diversity to a small number of the highest yielding cultivars.

The plant physiologist analyses the individual life processes and develops an understanding of the plant's response to fluctuations in environmental conditions. In other words, the plant physiologist aims to answer the following questions: How is it functioning? Why does it function in this way? Thus, he occupies the branch of plant sciences, which could help others to interpret and explain their obtained data. Therefore, the plant physiologist should become a vital partner of the experienced breeder in the process of developing and evaluation of new promising cultivars. For example, Polish breeders effectively exploited interrelationship between leaf area and yielding of cereals offered by a plant physiologist (Nalborczyk and Czembor 1990, Nalborczyk *et al.* 1986).

Generally, so far, the cooperation between breeders and plant physiologists is still poor, not fully explored and should be substantially improved. Probably, its weakness stems -among other things- from a lack of awareness amongst breeders concerning current advances in plant physiology. Recently, a considerable development in plant physiology was observed, resulting in important advances of "agrophysiology". This was achieved due to the availability of modern computerized portable devices and instruments, which enable the plant physiologist not only to work in the laboratory but also in the field and get thousand of measurements in short period of time. Such devices allow monitoring nearly complete chain of plant life processes i.e. starting with e.g. GMO (Genetically Modified Organism), physiological analysis through experiments in the laboratory and finally followed by field verification (Starck and Niemyska 1998) on the canopy scale (Pietkiewicz *et al.* 2002,Wyszynski *et al.* 2002).

In this article, the authors -as plant physiologists familiar with many plant breeding programs- would like to share with plant breeders, few

concepts related to physiological issues which seems to be crucial to the mutual cooperation between plant physiology and plant breeding.

SELECTION CRITERIA AND PLANT PHYSIOLOGY

While reviewing the worldwide literature we found that, the number of registered tolerant or resistant cultivars obtained by plant breeders is relatively poor as compared to their documented researches. The most commonly selected (popular) traits to be used as criteria in plant selection programs were those of a morphological or a simple physiological nature related to the yield (final outcome) but not to the yielding (process of yield formation) ones. Our search in ISI Science Citation Index within 1993-2000 years for the literature related to "selection criterion" or "selection criteria" (Table 2) revealed that, some of criteria were established upon the recommendation of certain common classical methods used in plant breeding (Fernandez and Kuo 1993, Chilagane 1994, Piepho 1995, Preciado Ortiz *et al.* 1995, Bokyeong *et al.* 1995, Wisniewski and Zagdanska 1998, Jamjod and rerkasem 1999*,* Voltas *et al.* 1999*,* Kamali and Boyd 2000). Very few positions that directly related to plant physiology were found (Wu and Tao 1995, Srinivasan *et al.* 1996, Akazawa *et al.* 1997). The rest of the cited criteria were related to traits of common growth parameters estimation e.g. mean lesion length within taproots (Salter *et al.* 1994).

To analyze more thoroughly the contribution of various agricultural disciplines for plant breeding we have chosen the case of International Center for Agricultural Research in the Dry Areas (ICARDA) as an example. According to SCI, data related to ICARDA output, in the view of newly registered cultivars should be considered as satisfactory one. Table 3 reveals their success in registration of many new germplasm lines between 1994 and 1999. Nevertheless, nearly all of these cultivars were developed without substantial use of progress in plant physiology (Table 4).

Moreover, it was surprising when we found that, the most of the realized by plant breeders works in the frames of selection criteria establishment had been undertaken in stress conditions. It is in disagreement with what plant breeders were practicing i.e. selection for yield should be conducted under non stressed conditions (Ceccarelli *et al.* 1996).

Recently, plant physiology offers some new methods to characterize physiological and biochemical traits to be looked for through plant breeding programs. We assume, that in the past, many valuable plant physiology researches did not leave the laboratory because scientists did not know how to make better use of their obtained valuable results. On the other hand, the breeders have not been convinced enough by the form of data presentation adopted by plant physiologists. In the following part of this article we willsurvey some of the physiological parameters that we believe are noteworthy, deserve more investigation and should attract plant breeders' interest and concern. Below, we are going to underline some of issues, which we believe that exemplify useful selection criteria.

Year	Author	Trait or criterion of plant selection/breeding	Plant
1993	C.	Fernandez G., Kuo Stress tolerance index (STI) = (potential yield \times stress $yield) \times (mean potential yield)$	Mung bean
1994	Salter R. et al.	Mean lesion length (vertical discoloration) within taproots (resistance to root rot caused by Fusarium species)	Alfalfa
1994	Okamoto M	Leaf color as indicator for chemical components of grain (N content)	Rice
1994	Chilagane A.	Farmers' criteria for evaluating: plant height, drought tolerance, threshability, milling qualities and cooking qualities	Rice
1995	Piepho H.	Desirability index	Various plants
1995	Worku T	Water-retention capability (flag leaf)	Wheat
1995	Wu P., Tao Q. N.	Physiological nitrogen use efficiency: efficiency in transferring nitrogen from terminal parts for grain production, HI and N content	Rice
1995	al.	Yagbasanlar T. et Biomass yield and harvest index (selection for improving seed yield)	Wheat
1995	Jackson P. <i>et al.</i>	Sugar yield or net merit grade	Sugar cane
1995	Preciado-Ortiz R. et al.	Growth and yield modeling as methods (shorter vegetative and longer reproductive periods has greater predicted yield)	Maize
1995	Johnson R.C. et al.	Shoot weight and HI have better correlation to yield than WUE and it relation to photosynthesis	Lentil
1995	Jafari S. et al.	High grain yield under salinity stress is better selection criterion for tolerance than biomass yield, harvest index	Wheat
1995	Pyl'nev V.	Number of fertile tillers/unit area	Wheat
1995	Kertikova D., Georgiev Z.	Regrowth rate: tolerance to intensive harvesting	Lucerne
1995	Bokyeong K. et al.	Chained ring weight (CRW) to select lodging resistant genotypes	Rice

Selection criteria found in the World-wide appraisal of plant breeding programs (ISI Science Citation Index).

Table 2

Table 2

Table 3 **ICARDA registration of new germplasm lines 1994-2000 (Information are collected from ISI Science Ci-tation Index)**

Year	Plant	Tolerance or resistance	
1994	Chickpea: eight germplasm	<i>Ascochyta</i> blight resistance, early maturing, and large seed	
1995	Barley: 'Giza 125' and 'Giza 125'	Moderate tolerance for high stress environments and diseases	
1996	Chickpea: Ladiz ILWC 292	Chickpea cyst nematode	
1996	Chickpea: (three germplasm lines)	Leafminer	
1996	Lentil: ILL 5582	Seed yield	
1996	Lentil: ILL 5588	Vascular wilt and <i>Ascochyta</i> blight	
1996	Chickpea: FLIP 87-59C	Drought	
1996	Chickpea	<i>Fusarium</i> wilt	
1997	Chickpea: FLIP 91-178C, FLIP 93-53C, FLIP 93-98C	<i>Ascochyta</i> blight, <i>Fusarium</i> wilt, and cold	
1997	Chickpea	Callosobruchus chinensis	
1999	Lentil [,] 'Barimasur-2'	Uromyces fabae [U. viciae-fabae]	
1999	Lentil: 'Barimasur-3'	Uromyces viciae-fabae and Stemphylium botryosum [Pleospora herbarum]	
1999	Lentil: 'Barimasur-4'	Uromyces fabae [U. viciae-fabae]) and Stemphylium botryosum [Pleospora herbarum]	
1999	Faba bean: `Fiesta VF'	Disease resistance (moderately susceptible to Botrytis fabae and moderately resistant to Ascochyta fabae).	

Table 4

ICARDA selection criteria for plant breeding (ISI Science Citation Index)

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Continued

Table 4

GAS EXCHANGE OF PLANT

Photosynthesis is the process by which energy is harvested from the sun and converted into plant biomass. The leader work of Nichiporovich *et al.* (1961 and 1967) established that, the main photosynthetic indices of plant productivity are: photosynthetic rate, photosynthetic area, leaf area duration, coefficient of photosynthetic plant efficiency and harvest index (Nichiporovich, 1997; Nichiporovich *et al.* 1961).

Estimation of gas exchange parameters allows some comparisons of leaf gas exchange between different genotypes (Choluj *et al.* 1997, Brestic *et al.* 1996, Choluj and Kalaji 1995). Also, it helps to study the effect of various unfavorable growth conditions, as e.g. effects of $CO₂$ enrichment on field crops, seasonal trends in photosynthesis rates, effect of insecticides, and assessment of herbivore impact on field crops as well as photosynthetic response to nutrient variation (Climate Stress Lab 1997). There is no doubt that it is a very simple and proper tool to underline crop fidelity and/or reliability through breeding programs.

The set of gas exchange parameters involving: photosynthetic rate (P_N) , transpiration (E), their ratio (WUE - water use efficiency), intracellular CO2 concentration (C_i or g_m), stomatal conductivity (g_s) and dark respiration (R_D) could be a very suitable selection criteria since they may reveal the potential of plant growth capacity (Kalaji and Nalborczyk 1991, £oboda *et al.* 2000). Estimation of dark respiration (R_D) as well as photorespiration $(R₁)$ is also important since these processes consume a big part of the photosynthesis products creating yield (e.g. legumes 30%, Nalborczyk *et al.* 1986). There are several commercially available systems for measurement of gas exchange parameters. The use of such computerized portable instruments enables us to realize proper measurements and get high throughput of recordings in both laboratory and field conditions.

It is important to underline here that, plant gas exchange parameters are very important tool to explore the level of the hidden photosynthetic potential of plants. Cereal breeders started to be interested in such traits for high and stabilized yield e.g. triticale yield which was increased by one of the most famous world wide triticale breeders- Prof. Wolski (Nalborczyk and Czembor 1990). Taking into account the possibility of producing by biotechnology a triticale plant with resistance to the herbicide BAAS (Dobrzanska *et al.* 1997), this crop possesses an opportunity to prevail in some aspects above other cereals in yielding. Indeed, recently triticale plant proved to show better performance on field till now occupied by other cereals.

CHLOROPHYLL *a* FLUORESCENCE

The performance of photosynthesizing plants might be an indicator for biological yield prediction (Donald 1962). It is well established that plants can usually exploit only about 5% of the solar energy, and produce assimilates by the absorption of visible light in the 400-700 nm waveband (Photosynthetically Active Radiation PAR). Excess energy must be dissipated mainly as heat and small part as an optical reemission, which is called "chlorophyll *a* fluorescence". Measurement of chlorophyll *a* fluorescence seems to be one of the best indicators of photosynthetic efficiency. This procedure satisfies the demand for rapid, non-invasive screening tests, and can be easily applied to assay the influence of environmental effects on physiological state of plants (Govindjee 1995, Strasser *et al.* 2000).

Available chlorophyll fluorimeters are based on two different measurement techniques. The first one is referred to as continuous excitation whereby a high intensity beam of excitation light induces chlorophyll *a* fluorescence emission from the sample and is immediately detected by special detector. The advantage of this technique is the rapidity of measurement at high time resolution. A typical measurement of one-second duration provides a lot of information related to the transient fluorescence induction curve, which is observed when a plant is strongly illuminated after at least 30 min. of darkness. This allows large scale screening of crops at throughput rates of many hundreds of samples per hour (Strasser *et al.* 2000).

The second type of chlorophyll fluorimeter uses a technique called Pulse Modulation to discriminate fluorescence emissions even in ambient light (natural conditions). Measurements with such instruments are generally performed over longer time periods and at a range of light levels. They can be used in combination with gas-exchange measurements to simultaneous monitoring both effect of $CO₂$ assimilation and chlorophyll *a* fluorescence in different conditions (Schreiber *et al.* 1986).

In stress conditions, the ability of a plant to use solar energy for photochemistry is reduced and hence an increase in dissipation of energy via chlorophyll *a* fluorescence is observed. Stress conditions will strongly influence the kinetics of this fluorescence signal in susceptible plants allowing the plant physiologist to quantify stress even before visible symptoms are observed. It is therefore not surprising that chlorophyll fluorimeters are often marketed under names such as "'Plant Efficiency Analyzer" or "Plant Stress Meter" as these devices are ideal for the rapid determination of stresses. The use of such parameters by plant breeders will allow them to get a rapid snapshot of the changes in plant photochemical efficiency experienced by the plants growing under different growth conditions in the field.

TOPOGRAPHY OF PHOTOSYNTHESIS

Some well known methods or techniques such as the use of isotopes, which enable not only the estimation of plant gas exchange but also the distribution of the assimilates in different parts of the plant have also

been intensively used. Carbon isotope ${}^{14}C$ methods enable estimation of the photosynthetic activity of shoots, shoot radioactivity decrease (respiration) and distribution of ${}^{14}C$ -labeled assimilates both in the shoot and root (Nalborczyk et al. 1981, Starck 2003, Gawrońska 1998, Dzierżyńska 1990).

In the 1981's a team led by Nalborczyk showed that, the exposition by isotope 14 C of five cereals (barley, oat, rye, triticale, and wheat) gives the opportunity to establish the photosynthetic topography of these genotypes and identified three types (models) of plant photosynthetic potential (Fig. 1): Leaf (barley, triticale and wheat), leaf-spike (barley and oat) and culm (rye). These models allow plant breeders to improve the efficiency of solar radiation energy captured by plant under field conditions (Nalborczyk and Czembor 1990). It was one of the best classical works of how plant physiology may help in plant breeding (Nalborczyk et al. 1981, Nalborczyk and Sowa 2001).

Fig 1 Main photosynthetic models affecting productivity of cereals (Nalborczyk 1998)

TRANSLOCATION OF ASSIMILATES

Translocation is the transport of the products of photosynthesis from leaves (source) to areas of growth and storage (sink). It involves phloem loading and unloading, assimilates allocation and partitioning (Niemyska 1986). If we understood different strategies of plants growing under various conditions it would be essentially advantageous for plant breeding (Starck *et al.* 1994, Starck 1995, 1996, 1997, Weems *et al.* 1999, Reynolds *et al.* 2001, Sowinski 2001, De Costa and Shanmugathasan 2002). Translocation of assimilates is the factor that should be considered as an important criterion for plant selection which determines yield by controlling how and where the products of the photosynthesis will be shared (Choluj *et al.* 2001). Furthermore, the relation between source and sink could offer better understanding of the plant growth strategy. Many factors are involved in this process e.g. source and sink activity, their size, and strength (Warren-Wilson 1986).

Knowledge of nutritional relations and hormonal balance of plants, could also add some important information to be used during the establishment of useful criteria for plant selection.

PLANT GROWTH ANALYSIS

Using combined parameters involving plant assimilatory surface and/or whole plant mass can enable to estimate the processes of growth and production of dry matter and its partitioning. The most used indices in the case of single plant analysis are: Relative Growth Rate (RGR), Unit Leaf Rate (ULR), Leaf Area Ratio (LAR), while the most used ones in the case of crops analyses are: Crop Growth Rate (CGR), Net Assimilation Rate (NARc), Leaf Area Index (LAI) and Leaf Area Duration (LAD) (Watson 1952, Radford 1967, Evans 1972, Pietkiewicz 1985, Hunt 1990, Pala and Pietkiewicz 2002).

The strategies of growth and yielding revealed with this method were described for nearly all important crops *e.g.* triticale (Czerednik and Nalborczyk 2000), wheat (Kornatowska and Pietkiewicz 1991), barley (Kalaji and Pietkiewicz 1993), rye (Nalborczyk and Sowa 2001), amaranth (Chwedorzewska and Nalborczyk 1994, Gontarczyk 1996), potato (Czerwinska *et al.* 1996, Olszewski *et al.* 1999, Pietkiewicz 1984, 1990, 2000) and rape (Zawadzki *et al.* 2000).

On the crop scale, LAI seems to be a crucial parameter characterizing the size of the plants (Watson 1947, 1952, Pietkiewicz 1985). One can find it in both simple analytical investigations as well as in mathematical modeling of growth, development and yielding of crops (see the pleiad of models CROPGRO, SUBGRO, DSSAT and their relatives in Bonhomme *et al.* 1996). Quite recently developed LAI meters enable systematic and massive recording of LAI for various plant species and canopy architecture analysis (McPherson and Peper 1998). Many scientific comparison works (Wilhelm *et al.* 2000) proved that, these instruments are useful for both plant physiology and breeding.

INTERRELATIONSHIPS BETWEEN MEASURED PHYSIOLOGICAL PARAMETERS

The application of correlation tests on some previous mentioned traits enables us to understand plant growth strategies under various growth conditions (Brestic *et al.* 1996). During an experiments conducted by Kalaji (1993), investigation realized with 8 barley cultivars of different geographical proveniences revealed that, when plants were subjected to stress condition caused by an excess NaCl salt (Figs. 2-3) plant growth strategies were shifted in different manners.

Fig. 2 The relationships between gas exchange parameters (photosynthetic rate P, water use efficiency WUE, intracellular CO2 concentration \overline{C} , stomatal conductivity g and dark respiration R) of barley seedlings under control (solid line) and saline condition (dashed line). Stars indicate significance level of correlation coefficients (* =0.95, **=0.99)

It can be observed (Fig. 2), that most of relations between gas exchange parameters were shifted and the tolerant barley cultivars' reactions were toward assuring new strategy, which is considered to be more economic in the view of water using (compare WUE values for control and stress conditions). Under control conditions, the relation between photosynthesis (P_N) and intracellular CO^2 concentration (C_i) was inversely proportional, but under salt stress it became directly proportional. This indicated how the photosynthetic apparatus was partly blocked to minimize the growth rate (survival rate), which is under normal conditions allied with more opened stomata and more loss of transpired water (see the values between stomatal conductance g_s – Water Use Efficiency WUE and P_N – WUE). The relationships between gas exchange parameters and growth indices of barley seedlings grown under saline conditions showed how the photosynthesis and respiration processes became more crucial for plant growth especially to that of shoot dry matter (Fig. 3). Generally, the relation of the above mentioned parameters, were either not shifted, strengthened, or inverted (Figs. 2-3).

Fig. 3 The relationships between some gas exchange parameters (photosynthetic rate P and respiration R) and growth indices (Relative Growth Rate RGR, Unit Leaf Rate ULR, Leaf Weight Ratio LWR, Shoot Dry Matter DM and Root/Shoot Ratio R/S) of barley seedlings under control (solid line) and saline (dashed line) condition. Stars indicate significance level of correlation coefficients ($* = 0.95$, $** = 0.99$)

Basing on found relationships, Kalaji (1993) proposed the following conclusions related to some traits that could be used as criteria of selection and determination of plant tolerance on salt stress:

- 1. The economy of plant growing without salinization is characterized by wide opening of stomata, builds up an excess of assimilatory organs which consumes a lot of photoassimilates for maintenance, gathering of K⁺ ions in the shoot, and even influences distribution of mono and bivalent ions.
- 2. This economy is changed under saline conditions into an efficient state which characterizes with: partially closed stomata, counterbalanced nutrient absorption demands, shifted water status, gas exchange processes and osmotic stress increase to prevent injury caused by absorption of toxic ions. It is achieved by maintaining only the size of assimilatory organs required to sustain limited growth of the whole organism. Besides, shifting the general investment of dry matter for root build up, using the available pool of non-toxic ions (K^+, Ca^{2+}) for osmoregulation and strengthening the embedded in cell membranes protective barriers against unfavorable influx of toxic ions ($Na⁺, Cl$) as well their further penetration. The timing of initial reactions is very important.

3. The screening for plant tolerance to salinity should be based on the following characteristics: growth pattern (using growth analysis), gas exchange, calculated water use efficiency, ion relationships and their accumulation in different plant organs. Then, there should be done the evaluation of selected physiological characters .

PLANTS STRESS AND YIELDING

In the context of plant breeding, from the point of view of plant physiology it is very important to discuss some items, which we think are invaluable to understand and establish our concepts of criteria selection.

Very important is the understanding of "yield fidelity", which is strictly related to the concept of sustainable agriculture. Looking for the stability of the yield means the ability to pay the cost of survival in the field. We should not look only for the high yielding plants but also the ones, which would pay less cost when harsher growth conditions are experienced. Plant breeders should be aware that plants growing under field conditions never experience the optimum (control) conditions. Usually, plants undergo moderate multi-stress (Starck 1997). Furthermore, we have to remember that the optimum for plants is not necessary to be similar to that of human beings. In most cases, plants shift all the physiological processes which can make their further existence possible what means that yield is not important in such a case (Kalaji and Nalborczyk 2000, Pietkiewicz 2000).

Yield stability mechanisms fall into four categories: genetic heterogeneity, yield component composition, stress tolerance and capacity to recover rapidly from stress. In the context of stress tolerance, it has often been found that ecological adaptation and agronomic objectives are different and can be mutually exclusive. However, if the yield is our interest, of plant tolerance should be estimated at all levels of plant organization from organelles to crop.

When plant is stressed, the results of acclimation, adaptation changes or damage will appear. This will be depended on duration of stress and its strength. It is very important to distinguish between what are the results or the reasons and to identify the "stress sequences cascade". Information about resistance can be easily received by screening of genetically diverted material (e.g. landraces and breeding lines) as a result of cooperation between physiologists and breeders (Flowers and Yeo 1995). They suggested that it would be performed as follow:

- Expose a range of genotypes to a specific stress and choose the ones that perform best as a crop under those conditions (plant physiologists).
- Beyond evaluation of existing genotypes, screening could be carried from within a composite cross-aimed at increasing the recombination between resistance and agronomic traits (plant breeders).

- Selection should be first for tolerance/resistance, then agronomic characters. This sequence is very important because our reliance on "agronomic-first" selection is strongly against potentially tolerant genotypes before they are fixed.

BREEDING METHODS AND PLANT PHYSIOLOGY

The earlier example of Prof. Nalborczyk's team cooperation (Nalborczyk and Czembor 1990) is really good evidence as to how plant physiology is able to indicate and underline some unseen new features to be looked for by the plant breeders. The results of Prof.Wolski's team works are one of the main selection bases of today used in the cooperation program between the Department of Plant Physiology – Warsaw Agriculture University and DANKO (well known Polish Breeding Center as donor of triticale to the whole world). The main goal of this cooperation is study on crop physiology of conventional and half-dwarfed winter triticale plants. Generally works dealing with plant breeding and traits selection should depend on: crop ability to turn solar energy into biomass production, canopy spatial structure (LAI), changes in Red/Far Red ratio, and chlorophyll fluorescence under various stresses. This will allow achieving better characterizing of modern plant physiological ideotypes (Donald 1968).

The work of £oboda and co-workers (£oboda *et al.* 2000) in order to evaluate maximum potential of dry matter yield for the leading brewing barley forms is noteworthy. Measure of parameters describing the rate of physiological processes involved in crop yielding (photosynthetic rate, dark respiration, LAI, PAR absorption, and many others) and considering them jointly allowed to characterize the "ideotype" of the crop production capacity of nearly 100 t of dry matter \times ha⁻¹. Obviously such a form, the same as the Donald's ideotype (Donald 1962, 1968) will never be get, because of the influence of some factors (fluctuation in PAR incoming during vegetation period, self-shedding of the leaves and other constraints) nevertheless it allowed to specify some important physiological traits affecting the yield.

We believe that plant physiology can play an important role in both conventional (Mendelian) plant breeding (pure line, mass selection, pedigree, bulk, back crossing etc.) and in the case of "genomic" revolution (tissue culture, protoplast fusion, and recombinant DNA and genetic engineering methods). Surely, "molecular" plant physiology will contribute to "molecular breeding" progress since it is also functioning already carefully at the cellular level (Ondrej 1997, Bohnert and Jensen 1996). Independently of which object "Plant" or "DNA" should undergo breeding processes, it also can help to understand the whole organism homeostasis (Yeo 1998). The use of the methods outlined above to check the physiological trait fixation allowed plant physiologists to take part in evaluation of performance of GMOs multiplied in the field.

Concluding the previously mentioned facts, concepts, and results, we would like to underline some remarks related to the cooperation of plant physiology and breeding:

- Plant physiologists are well prepared to meet the needs of development of plant breeding due to adequate methods and devices used for screening the proposed selection criteria.
- Plant geneticists, breeders, and physiologists may work in concerted action. This would enable them to better understand the physiological- biochemical processes and their interactions, which determine the tolerance and the produced yield.
- Gas exchange measurements, chlorophyll *a* fluorescence screening, classical growth estimation, and isotopic exposure can help to reveal the hidden yield potential of crop genotypes.
- The preliminary selection of plants should be individual for yield, quality, or stress tolerance since its combination is elusive and selection for one magnitude is still at the cost of the others. Furthermore, we should distinguish where the crop is for trade or for subsistence.

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