Analysing the physiological effects, and developing simple protocols for high throughput automatic determination, of seedling and spike densities to provide support to selection in breeding programs

A joint project proposal by the Plant Phenomics (UB) and Crop Physiology (UdL) Groups of AGROTECNIO in collaboration with Semillas Batlle (a SME family-owned seed company)

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Brief Introduction

In wheat and barley, seedling density may be relevant for determining radiation interception and therefore growth, particularly during early phases of crop development (and in general with short-season crops). Early vigour (leaf area produced early in the season) could be an important trait for cereals as it may affect early evapotranspiration which might affect yield in different ways (it may reduce the fraction of evaporated water and may create a canopy with more competitive ability against weeds but might also exacerbate terminal drought by depleting soil water earlier). Seedling density is a relevant component or early vigour, and yield does respond to plant density, but in a hyperbolic fashion. Naturally, differences in seedling density modifies the pattern of tillering which may be important if later initiated tiller are considered wasteful (as they would likely be sterile). Finally, a major consequence of seedling emergence and early vigour may be the number of fertile spikes that the crop produce.

Physiologically it is not clear yet (and there is debate in the literature on this) whether the production of excessive tillers would penalise yield and should be avoided and to what degree the differences in spikes per unit area is relevant for establishing yield differences between elite germplasm (when it has been documented that those genotypic differences in grains per m^2 are mainly related to grains per spike).

Concerning the automatic evaluation of spike density, based in the use of RGB or thermal images acquired from above the canopy, it only accounts for the spikes total or particularly exposed to the light (i.e., the upper-canopy spikes). The upper spikes belong to main and primary tillers and therefore these are the spikes contributing more to the grain yield. In fact, the spike density evaluated automatically correlates better against grain yield than the manual counting which accounts for all the spikes present in the canopy, including the lower, smaller, and mostly infertile spikes. In that sense differences in spike density, whatever the cause (sowing density, genotype, crop duration, fertilization) may affect both the performance of the automatic counting and the portion of lower spikes not accounted) together with their correlation with grain yield.

Empirically, it is important to count with tools that allow the breeders to know the seedling density, early vigour, and spikes per m^2 in each of the plots to (i) select for some of these traits, and/or (ii) weigh yield differences between plots that might be due to differences in seedling density more than true genotypic ones (and therefore may produce a bias in the selection process).

Major aims

In this context it would be relevant for adjusting the selection process of a breeding programme to have both (i) a quantitative knowledge of the possible effects of seedling density, early vigour, and spike density at flowering on the yield performance, and (ii) a tool available for quickly and easily determining seedling density, early vigour, and spike density at flowering in hundreds of plots. This small project aims to cover these two elements.

Regarding the quantitative knowledge of the possible effects, the Crop Physiology group will be responsible for conducting specific experiments as well as measuring these particular attributes in wheat and barley plots of Semillas Batlle aiming to (i) establish relationships between seedling and spike densities, as well as between each of them and grain yield, (ii) understand the physiology behind these relationships.

Regarding the production/improvement of tools, the Plant Phenomics group will lead the process of (i) taking RGB (Red Green Blue) and thermal images from ground and aerial (UAV) platforms and (ii) creating/improving the software to transform the images into quantitative assessments of the variables analysed, (iii) developing simple protocols for breeding companies to use in their selection fields.

Brief description of planned work

Different sowing densities, and genotypes of wheat and barley will be tested. Remote sensing evaluations on seedling and spike densities will be compared with manual counting's. In addition, grain yield will be evaluated at maturity.

Regarding the quantitative knowledge of the possible effects, the Crop Physiology group will carry out dedicated field experiments in which genotypes of wheat and barley will be subjected to different seedling densities and within 1-2 of these densities modifying artificially the spike density at heading (altering manually tillering dynamics), measuring throughout the growing season the dynamics of tillering and radiation interception and determining the reproductive output resulting from the process, determining spike fertility at flowering, grain abortion, and grain weight together with indirectly estimating rates of canopy senescence. Similar measurements will be taken in selected plots of Semillas Batlle. These detailed treatments (dedicated experiments) and some of the measurements (dedicated experiments, selected plots in Semillas Batlle program) will also serve for the Plant Phenomics group in its aim of producing/improving tools to make it realistically possible to accurately estimate, in an automatic manner, seedling and pike densities in actual breeding programs.

Regarding the production/improvement of tools, the Plant Phenomics group will organise field visits, coordinated with the Crop Physiology and Semillas Battle groups, at the specific key stages that are deemed optimal for the capturing image data of the trials put forth by the group. These stages will be soon after seedling emergence for seedling density and early vigour, and during anthesis and grain filling for spike density assessments. Thus, at least 2-3 field visits of the Plant Phenomics group are anticipated per project year. In addition, the Plant Phenomics group will update the CerealScanner software, and the data collected will be compiled into that updated version of the software with added support for seedling density and UAV-based image assessments. Manually measured data taken by either the Crop Physiology or Plant Phenomics groups for actual density of seedlings, early vigour and spikes per unit land area will be used by the Plant Phenomics group for improving and/or validating the algorithms required. In addition, data of crop yield taken by the Crop Physiology group in the specific experiments conducted by them and by Semillas Batlle in their breeding plots will be used to assess correlations between productivity and seedling and spike counting (both automatically and manually).

Finally, recommendations will be jointly produced by both Groups of AGROTECNIO for breeding companies (i) for the use of tools for assessing seedling and spike densities in a high-throughput manner compatible with having to assess hundreds of plots, and (ii) for the relative importance of these aspects to be considered when identifying superior genotypes in the selection process (to avoid eliminating a valuable offspring due to problems in the plot and *vice versa*.

Capacities and adequacy of the two groups

Plant Phenomics Group

The different remote sensing approaches will build on the early work conducted by the Plant Phenomics group in the development of the CerealScanner software https://integrativecropecophysiology.com/software-development/cerealscanner/), which currently supports some of these specific crop assessments for ground but not aerial image data assessments (https://integrativecropecophysiology.com/software-development/cerealscanner/, https://gitlab.com/sckefauver/cerealscanner).

Specifically the Plant Phenomics group has pioneered the development of automatic spike counting under field conditions (and without the use of supporting systems, such as artificial lights, filters) basically using RGB (reed-green-blue) images, acquired from ground, using a pole (Fernandez-gallego et al. 2018a,b) or with the sensor placed in an unmanned aerial platform (Fernandez-Gallego et al. 2020). In addition, thermal images are also amenable for ear counting and false color makes quantification more straight forward (Fernandez-Gallego et al. 2019a,b) even if limited with environmental conditions (need of sunny days) and the comparatively low resolution of the thermal camera. While all the above publications on spike counting have been made on wheat, the Group has also experience in barley through a former collaboration with Syngenta publication.

Fernandez-Gallego, J.A., Kefauver, S.C., Aparicio-Gutierrez, N., Nieto-Taladriz, M.T., **Araus, J.L.**, 2018a. Wheat ear counting in-field conditions: high throughput and low-cost approach using RGB images. *Plant Method*s **14**:22.

Fernandez-Gallego, J.A., **Kefauver, S.C.**, Gutiérrez, N.A., Nieto-Taladriz, M.T., **Araus, J.L.**, 2018b. Automatic wheat ear counting in-field conditions: simulation and implication of lower resolution images. In *Remote Sensing for Agriculture, Ecosystems, and Hydrology XX* (Vol. 10783, p. 107830M). International Society for Optics and Photonics.

Fernandez-Gallego, J.A., Buchaillot, M., Aparicio Gutiérrez, N., Nieto-Taladriz, M.T., **Araus, J.L.**, **Kefauver, S.C.**, 2019a. Automatic wheat ear counting using thermal imagery. *Remote Sensing*, *11*, p.751.

Fernandez-Gallego, J.A., Buchaillot, M.L., Gracia-Romero, A., Vatter, T., Vergara Diaz, O., Aparicio Gutiérrez, N-. Nieto-Taladriz, M.T., Kerfal, S., Serret, M.D., **Araus, J.L.**, **Kefauver, S.C.**, 2019b. Cereal crop ear counting in field conditions using zenithal RGB images. *JoVE (Journal of Visualized Experiments)* 144 e58695, doi:10.3791/58695 (2019).

Fernandez-Gallego, J.A., Lootens, P., Borra-Serrano, I., Derycke, V., Haesaert, G., Roldán-Ruiz, I., **Araus, J.L.**, **Kefauver, S.C.**, 2020. Automatic wheat ear counting using machine learning based on RGB UAV imagery. *The Plant Journal* **103:**1603-1613.

Crop Physiology Group

The researchers of the crop physiology lab have a long-standing record of contributions to knowledge in terms of yield physiology as affected by environmental, management and genetic factors. In particular, over the last decade or so the group has pioneered initiatives for studying the reproductive output of cereals when affected by genotypes x environments (Elía et al., 2018; García et al., 2019; Prieto et al., 2020; Ochagavia et al., 2021), including the study of alterations in tillering dynamics (Ferrante et al., 2013).

Elía, M., **Slafer, G.A., Savin, R.,** 2018. Yield and grain weight responses to post-anthesis increases in maximum temperature under field grown wheat as modified by nitrogen supply. *Field Crops Research*, **221**: 228-237.

Ferrante, A., **Savin, R., Slafer, G.A.,** 2013. Floret development and grain setting differences between modern durum wheats under contrasting nitrogen availability. *Journal of Experimental Botany*, **64**:169-184.

Ferrante, A., **Savin, R., Slafer, G.A.,** 2020. Floret development and spike fertility in wheat: differences between cultivars of contrasting yield potential and their sensitivity to photoperiod and soil N. *Field Crops Research*, **256**: 107908.

Garcia, A.L., **Savin, R., Slafer, G.A.,** 2019. Fruiting efficiency differences between cereal species. *Field Crops Research*, **231**: 68–80.

Ochagavia, H., Prieto, P., **Savin, R., Slafer, G.A.,** 2021. Developmental patterns and rates of organogenesis across modern and well-adapted wheat cultivars. *European Journal of Agronomy*, **126**: 126280.

Prieto, P., Ochagavia, H., Griffiths, S., **Slafer, G.A.,** 2020. Earliness per se x temperature interaction consequences on leaf, spikelet and floret development in wheat. *Journal of Experimental Botany*, **71**: 1956–1968.

Furthermore, both groups have successfully collaborated since long time ago with several papers published in prestigious journals (as well as in books and book chapters by recognised international publishers). Just to illustrate the length and depth of such successful collaborations the first and last of the papers signed by the IPs of both groups were published in 1999 (*Aust J*

Plant Physiol **26**:345-352) and 2020 (*Field Crops Res* **254**:107827), and these collaborations included the combination of detailed physiology and HTP measurements (e.g. *Ann Appl Biol* **150**: 253-257; *Europ J Agron* **73**:11-24).

Estimated Budget, EUR

Naturally, the funding available in this call will only partially cover the actual needs of the project. Both groups of AGROTECNIO will cover the substantial additional costs that the project will have from own funding from other sources. What we require from this specific call is mainly funding to cover part of the personnel costs the project will have, as well as a minimal share of the required funding for field and lab costs, the basic expenses for visiting the experimental fields and a small share of the equipment that will be used.
Please find below the specific funding requested to this call.

	Plant Phenomics (UB)			Crop Physiology (UdL)			**TOTAL**
	Year 1	Year 2	**TOTAL**	Year 1	Year 2	**TOTAL**	
Personnel[0]	2,500	2,500	**5,000**	2,400	2,400	**4,800**	**9,800**
Field and lab costs[1]	500	800	**1,300**	1,150	1,150	**2,300**	**3,600**
Travel[2]	450	450	**900**	450	450	**900**	**1,800**
Equipment[3]	4,000	800	**4,800**	0	0	**0**	**4,800**
Indirect costs*	0	0	**0**	0	0	**0**	**0**
Total	7,450	4,550	**12,000**	4,000	4,000	**8,000**	**20,000**

[0]Casual technicians (scholarships/contracts)
[1]Materials used to install, maintain, and sample experimental fields.
[2]Travel to fields and meetings between partners.
[3]Mavic 2 Enterprise Advanced RGB+TIR camera 50% cost sharing with UB, total 9600 euros.
*It was assumed that there will be NO indirect costs charged to the project.