Issues about crop systems durability, environment protection, and input reduction are in the center of the discussions concerning future agro-systems. In this context, variety mixtures, arouses a growing interest. To date, studies of varietal mixtures have only remained descriptive and post-harvest made, making it impossible to predict mixing effects of given varietal mixtures. Thus, the development of varietal mixtures having both relevant properties for the development of a more sustainable agriculture and interesting agronomic performances requires to be able to disentangle the mechanisms driving interactions between genotypes. To clarify the contribution to each genotype in a given mixture, nondestructive and noninvasive methods are highly desirable. Hyperspectral images (NEO HySpex VNIR-1024, 400-1000 nm, 216 bands) have been acquired in the field, 1 meter above wheat plots that were constituted by either a single variety or varietal mixture at several growing stages. The purpose is to discriminate the pixels of vegetation according to its variety thanks to its spectrum. After filtering out the soil pixels, the discrimination was carried out on leaves pixels by means of an original combination of Partial Least Squares – Discriminant Analyze (PLS-DA) and Support Vector Machine (SVM). Different pre-treatment and strategies of Calibration-Validation sampling are tested. In some conditions, the pixels of the validation set were classified in the correct variety up to 90%. The spatial distribution of the quality of prediction is investigated. Our work also showed that the discrimination model works better with the most illuminated leaves. These results show promising performances which could lead to a use of this methodology to quantify biomass production of variety mixtures. This plants characterization tool could be used in plant improvement programs and research on variety mixtures.