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Visualizing harvest index in crops**Agnieszka Wnuk^{1*}, Andrzej G. Górny², Jan Bocianowski³, Marcin Kozak^{4,5}**¹ Department of Experimental Design and Bioinformatics, Warsaw University of Life Sciences – SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland.² Institute of Plant Genetics Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland.³ Department of Mathematical and Statistical Methods, Poznań University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland.⁴ Department of Botany, Warsaw University of Life Sciences – SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland.⁵ Department of Quantitative Methods in Economics, University of Information Technology and Management in Rzeszow, Sucharskiego 2, 35-225 Rzeszów, Poland.

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ABSTRACT

Harvest index describes plant capacity to allocate biomass (assimilates) into the formed reproductive parts; hence it is an important trait for plant breeding. Its relationships with biomass and grain yield follow the multiplicative yield component model, in which grain yield is a product of harvest index and biomass yield. In this paper a visualization technique is proposed to give insight into associations among harvest index, biomass yield and grain yield. The technique consists of several types of plots, namely stripcharts, a scatterplot matrix, a new type of plot, the MM-Contour plot (which is a contour plot dedicated to a multiplicative model), and its trellis version. Contrary to methods of yield component analysis, which offer information about the influence of harvest index and biomass yield on grain yield, the plots enable one to concentrate on harvest index and draw conclusions about the patterns of its relationships with biomass and grain yields for various groups like cultivars or environmental habitats. The technique is presented using data for 32 field-grown wheat cultivars and its subset of seven cultivars.

Key Words: *grain yield; scatterplot matrix; winter wheat; MM-Contour plot; trellis plot.*

INTRODUCTION

In cereals, like in other crops, the continued improvements in grain and/or seed yields are associated with numerous endo- and exogenous factors. Among them, various interrelated morpho-physiological mechanisms contributing to an enhanced allocation of biomass (plant assimilates) to the reproductive plant parts appear to be crucial. During several decades, this plant feature was successfully exploited in most breeding programs worldwide, and its modifications led to different patterns of allocation of dry matter between crop cultivars hitherto released (Hay, 1995).

In several crops, such as wheat or other cereals, the considerable progress in breeding for higher yields is achieved mainly through man-made selection forces for the harvest index (HI), that is, an increased plant capacity to allocate biomass (assimilates) into the formed reproductive parts (Austin et al., 1980; Evans, 1993; Sayre et al., 1997; Calderini et al., 1999; Foulkes et al., 2007; Gutam, 2011; Mazid et al., 2013). Generally, the harvest index, which is the proportion of plant biomass allocated into grains (seeds), is the commonly known measure for the efficiency of the process and source-sink balance (Evans, 1993; Acevedo et al., 2002; Lawlor, 2002; Reynolds et al., 2007). Nevertheless, morpho-physiological examinations made by Austin et al. (1980) in a modern wheat collection suggest that the increased partitioning of the dry matter into grains already attained its physiologically justified limit (HI value of around 0.6) (Austin et al., 1980; Slafer et al., 2005). Reynolds et al. (2007; 2011) emphasize that the conclusions presented by the researchers in the 80s of the twentieth century are still valid. However, according to Fischer (2007) the today situation is not as dramatic as Austin et al. (1980) predicted, because at the moment this limit for HI (0.6) is not exceeded. However, there is no doubt that there is a need to pay close attention to alternative approaches to increasing the efficiency of plant yielding. Thus now more than ever it seems to be important to use modern plant breeding methods, which is why breeding for larger plant biomass (including focus on increase photosynthetic activity or radiation use efficiency (RUE)) has become one of alternative approaches if further genetic gains in wheat and others plants yielding are to be realized (Richards, 1996; Fischer, 2007; Reynolds et al., 2009; Reynolds et al., 2011). Nevertheless, one may expect that the range of genotypic variation in HI among the modern, short-statured wheat germplasms seems to be more and more limited, and the actually observed variation of this trait in the breeding collections appears to be affected by environmental factors more than before. Some researchers even believe that in modern breeding plant there is a tendency to lead the breeding process in the direction of not just HI, and even both HI and biomass, but at biomass itself (Aisawi et al., 2010; Fischer, 2011).

Therefore, analyzing harvest index and its associations with grain and biomass yields seems to be an important issue for various disciplines of plant biology, including plant physiology, genetics and breeding. This is, however, not a simple matter, because the triplet HI, biomass yield and grain yield is characterized by a specific set of associations, which in terms of HI are described by the following model:

$$HI = \frac{\text{grain yield}}{\text{biomass yield}} \quad (1)$$

Both yields are measured directly while the HI-value for a particular experimental unit is calculated based on Eq. (1). Note that Eq. (1) is a transformation of the following multiplicative model (Kozak and Mądry, 2006; Kozak et al., 2007):

$$\text{grain yield} = \text{biomass yield} \cdot HI \quad (2)$$

Such a multiplicative model assumes thus that the dependent (final) trait (here grain yield) is a product of its components, the multiplication factors (e.g. Sparnaaij and Bos, 1993; Piepho, 1995; Wang et al., 2012). In agronomy and plant breeding, in the model (2), grain yield is the so-called complex trait (Sparnaaij and Bos, 1993), while biomass yield and

HI are grain yield multiplicative components (Kozak and Mađry, 2006). Hence to analyze how grain yield is determined by biomass yield and HI, methods of multiplicative yield component analysis can be applied (Kozak et al., 2007). Various methods for yield component analysis have been proposed. Fraser and Eaton (1983) presented a complete account of these methods until 1983, while Kozak and Mađry (2006), Kozak et al. (2007) and Kozak and Verma (2009) mentioned methods that were proposed after 1983. All those methods attempt to characterize the influence of component traits on the complex trait, that is, grain yield in Eq. (2). Such an analysis for a particular genotype shows whether this is biomass yield or HI what influences grain yield stronger.

The above methods, however, do not offer too many insights that would facilitate interpretation of the studied full relations between those attributes. Here we show that visualization techniques can be of much help in such interpretation, and allow more complex approach for analysis of the studied model. Instead of merely asking whether it is biomass yield or HI that has the strongest influence on grain yield, we attempt to visualize the relationships, in that way supporting genotype-to-genotype (visual) comparison. The first attempt to use visualization techniques for analysis and presentation of the entire multiplicative model was reported by Wnuk (2013), who studied several graphical methods for better interpretation of this model. In our study, we will consider the model (2) with the focus on HI, and study how it behaves in various winter wheat genotypes. Even though the relationships seem very simple at first glance, visualizing them does not have to be that simple if we want the graphs to be really useful. We will show several visualization methods that could help one interpret the relationships among grain yield, harvest index and biomass yield. We also found that trellis visualization was great support for modified contour plot, especially when as many as 32 genotypes were to be analyzed and interpreted.

The aim of this paper is to propose visualization techniques to support interpretation of harvest index and its associations with grain and biomass yields for a more complex analysis. We will also compare different types of graphs that enable one to look at the model (2) at different levels, and also propose to use a trellis display of a MM-Contour plot (see below), which can be of help when many genotypes are studied.

MATERIALS AND METHODS

PLANT MATERIAL AND EXPERIMENTAL DESIGN

The technique proposed in the paper will be applied for the example for 32 cultivars of winter wheat. However, initially focus will be given to a subset of seven cultivars, whose some details are presented in Table 1. These cultivars were evaluated in the drill-sown field experiment conducted in 2006. The experiment was arranged as a randomized complete block design with 32 cultivars and advanced breeding lines and three plot-replications. The trial was carried out on the experimental fields of the Institute of Plant Genetics of the Polish Academy of Sciences in Poznań, Poland, with a moderately low content of plant-available N in the soil A-horizon (< 30 ppm N; by a modified Spurway's method; Nowosielski, 1974), with sandy-loam soils classified as the domestic IVb/V class. Each plot (1.70 m²; about 300 plants m⁻²) consisted of six 1.7 m-long rows spaced 18 cm apart. Reduced rate of N fertilizer (76 kg N ha⁻¹; added as ammonium nitrate in three sub-rates) was applied. Optimal rates of other fertilizers (superphosphate + K and Mg sulphates + trace elements) were also dressed. During the whole vegetation season, plots were irrigated by sprinkler to eliminate water shortages and optimise variations in rainfall distribution. Standard chemical treatments were used to control pathogens, insects and weeds. A special net was extended over the plots to eliminate plant lodging during reproductive growth phases. At harvest, dry weights of the separated vegetative and generative (grains) plant parts were determined by oven drying (65°C for 72h).

Table 1. Description of the chosen wheat cultivars together with their total plant biomass, grain yield and harvest index (HI) in the field experiment.

Cultivar	Release and origin	Notes	Field data (mean)		
			Biomass t ha ⁻¹	Grain yield t ha ⁻¹	Harvest index (HI)
Rapsodia	2002, UK	a modern pasture cv.; high yield potential; resistance to biotic stresses; semi-dwarf plants (80-85 cm); enhanced lodging resistance	8.41	4.68	0.56
Tonacja	2001, Poland	a modern bread cv. (C) with high yield potential; moderately tall plants (90-95 cm); resistant to lodging	9.48	4.80	0.51
TAM 110	1970/80's, USA	moderately tall (85-90 cm) cvs from the southern regions of USA; breed by the Texas	9.44	4.70	0.50
TAM 200	1970/80's, USA	A.&M.; potential sources of yield stability and resistance to water shortages and soil acidity (Al)	10.03	4.90	0.49
Astron	1989, Germany	a moderately tall (90 cm) bread cv. (A); potential source of grain quality, winter hardiness and resistance to abiotic and biotic stresses	9.31	4.45	0.48
Grana	1970, Poland	the first Polish semi-dwarf cv.; for a long time considered as a check for high and stable yielding; plant height about 85 cm; cultivated till 1990'	10.62	4.88	0.46
MA77/97	1970's, USA	relatively high cv.; extensive type; potential source of grain quality and the resistance to biotic and abiotic stresses	12.31	4.89	0.40

VISUALIZATION TECHNIQUES

To support interpretation concerning HI, plant biomass and grain yield, we will draw the following plots:

(1) *Stripcharts*. Stripcharts provide a univariate picture of the traits' distribution within genotypes; hence they plot two-dimensional data, one dimension being the genotype and the other being the trait's value. Stripcharts should be constructed with genotypes on the y-axis and trait values on the x-axis. In that way, comparing genotype-wise distributions of the trait is easy. Here it is important to order the genotypes in a particular way; since our main interest lies on HI, the genotypes should be ordered by decreasing mean HI values, so that genotypes with the highest mean of HI—which can be considered the most desirable in breeding/genetic evaluations—are presented on the top of the plots. The stripcharts for the three traits are set one next to other, with that for HI being the first in the order. The stripchart for seven genotypes is presented in Figure 1.

(2) *Scatterplot matrix*. A scatterplot matrix (Cleveland 1985, pp. 210-213) is a set of bivariate scatterplots of the three (in our case) variables, arranged with shared scales. The genotypes are represented by different plotting symbols. In a scatterplot matrix, a row variable is represented on the y-axis while the column variable on the x-axis. By means of the scatterplot matrix one can picture a bivariate relation between each pair of variables; hence we might say that these plots show three dimensions—two are represented by x- and y-axes, and the third one by genotypes. A scatterplot matrix for seven genotypes is presented in Figure 2.

(3) *MM-Contour plot* (a contour plot dedicated to a multiplicative model). Note that the two above-mentioned plots are either two-dimensional (stripcharts, with a trait being one dimension and genotype being another) or three-dimensional (scatterplot matrix, with two traits and genotype) plots. Our data, however, are four-dimensional: three traits and genotype. Hence presenting them in two or three dimensions causes some losses in information. For example, in a scatterplot matrix one may have problems with linking points for a particular genotype from one panel to the points for this genotype from another panel; for stripcharts it is even impossible. Hence, we adapted the contour plot for the purpose of presenting data that follow the model (1); this plot can also be used without the fourth dimension represented by genotypes. By means of this plot one can see all the four dimensions for each particular observation. The idea for this plot was taken from Gelman et al. (2002), who plotted counts and rates of citations of various professions from the New York Times database. The difference between his idea and ours is that we base the contour plot on model (1) and we do not use logarithmic scales.

The plot is constructed in such a way that it represents the model (1), so – contrary to the other two plots – it takes into account the multiplicative specificity of the relationship (2). Grain yield is presented at the y-axis while biomass yield on the x-axis, while HI limits are represented by additional lines inside the plotting region (gray color). *MM-Contour plot* for seven genotypes is presented in Figure 3 and for the complete 32 genotype set in Figure 4.

THE TRELIS DISPLAY OF THE MM-CONTOUR PLOT

When there are many genotypes to present in one graph, previous types of plots do not work well because of too many plotting colors and symbols. In such instances a trellis plot can help, allowing one to analyse the data effectively and present a large number of genotypes. In this approach it is crucial, however, that all these plots be arranged with the same scales so that the plots could be easily compared and that all the plots be presented next to each other with the same x- and y-scales are used for each plot. This division is up to the researcher; it can be done based on some property of the genotypes, including origin, time of release and the like; alternatively, grouping can be done based on similar grain yield or HI – each such grouping may show different phenomena.

In general, each of these types of plots provides a different picture of the relationships studied. Stripcharts offer an immediate ordering of the genotypes in terms of HI, and in addition show whether this ordering is similar for the other two traits; the distribution of each trait is provided for each genotype. Scatterplot matrix offers the picture of bivariate relationships, which is very natural for human understanding of relationships. *MM-Contour plot* offers the most complete picture of three-variate relationships. Trellis plot helps in the case of many genotypes. In our case, to all these plots, an additional dimension incorporated by the genotypes is added. The visualization technique we present consists of the three plots analyzed together; each plot may offer interesting information. All graphs were performed in R (R Development Core Team, 2013).

RESULTS

ANALYSIS FOR SEVEN GENOTYPES

Although both grain and biomass yields were diverse in particular cultivars, the variability in HI was small, as demonstrated in the stripcharts (Figure 1). The greatest range of variation among plot values for HI was observed for TAM 200 (0.45–0.52) and Grana (0.43–0.49). The modern cv. Rapsodia clearly had the highest HI, while the extensive cv. MA77/97 the smallest. From Figure 1 one can see that Rapsodia exhibited high HI values and small biomass yields, which contributed to medium grain yields. For MA77/97 the situation was the opposite, because HI values were small while biomass values were high; grain yields were quite similar to those of Rapsodia. Hence these two cultivars have rather opposite types of partitioning of assimilates to harvestable product (grains).

Note also the cultivar Astron with variable grain and biomass yields, but a very stable HI. A similar situation was observed in Tonacja. In turn, biomass yield in Grana was very little variable. Stripchart offers quite interesting information about the traits, but nothing about relationships among the traits.

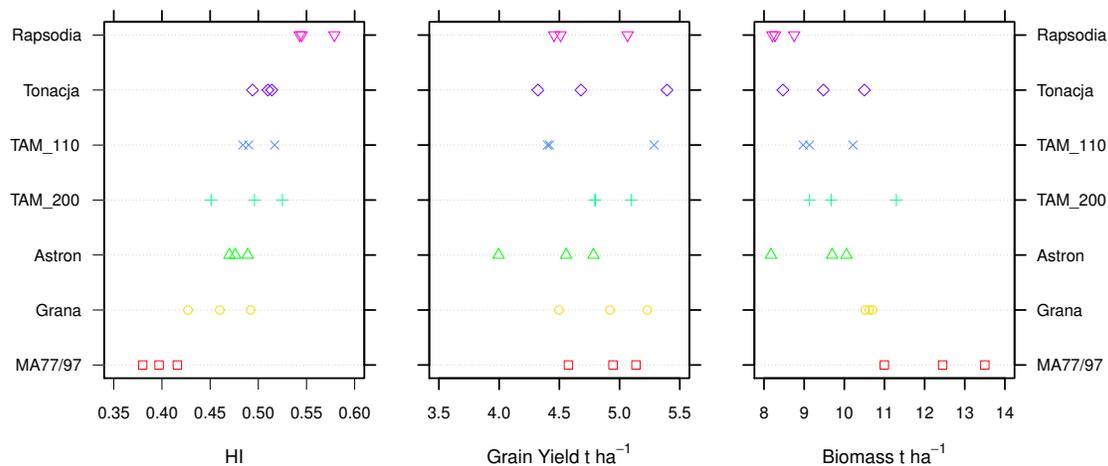


Figure 1. Stripcharts for harvest index (HI), grain and biomass yields among seven winter wheat cultivars (genotype in the graph were ordered by the mean value of the HI).

Scatterplot matrix (Figure 2) suggests that, when pooling the cultivars, grain yield was not related to HI, while showing quite a strong linear positive relationship with biomass yield. Also HI and biomass yield were linearly though negatively related. However, when looking into particular cultivars, this conclusion does not have to hold. Note for example the relation between HI and grain yield in Grana: there was a clear linear positive relation. In MA77/97 this relation also existed, but was negative. No such relation could be observed in Astron. In general, the across-genotype relations were often opposite to genotype-wise ones (Kozak and Verma, 2009 discuss the difference between across-genotype and genotype-wise yield component analyses).

MM-Contour plot (Figure 3) summarizes these observations, in addition offering some more information within a single plot. Again it can be easily seen that Rapsodia exhibited the highest while MA77/97 the smallest HI values – in general the closer the genotype is to the top-left corner of the plot, the higher HI it had. One can see that the observation in Rapsodia with the highest HI (0.54–0.58) also exhibited high grain yield (4.46–5.66 t ha⁻¹) and the lowest for biomass yield (8.21–8.75 t ha⁻¹); this could also be seen from scatterplot matrix. We can much easier grasp the constancy over replications of HI – take the cv. Grana, for example. We can easily see that this cultivar exhibited unstable HI and very stable biomass yield, so the lack of constancy over replications in its grain yield was due to the lack of constancy over replications in HI. On the other hand, TAM 200 exhibited unstable HI and biomass yield, but quite stable grain yield. Astron represented yet another strategy, in which its HI was stable, whilst grain and biomass yields were not. We also noticed that genotypes with lower biomass, such as Rapsodia, usually have higher HI. Such information can be very useful for breeders, because a lower harvest index means a higher yield of biomass and thus a smaller share of the grain. MM-Contour plot uses the isometric scale (Sarkar, 2008), in which the unit of variable on the x-axis and the unit of variable on the y-axis are represented by the same physical length. If we look at Figure 3, it means that in Figure 3 the physical distance between the values 8 and 9 (t ha⁻¹) of biomass yield on the x-axis is the same as the physical distance between the values 4 and 5 (t ha⁻¹) of grain yield on the y-axis.

Such a procedure is justified only when the axis OX and OY represent variables with the same units whose direct comparison makes biological sense.

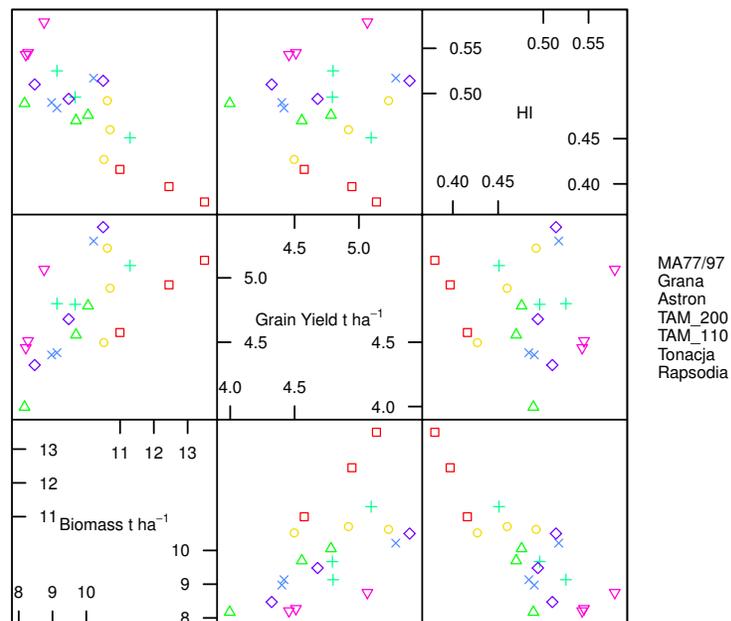


Figure 2. Scatterplot matrix for harvest index (HI), grain and biomass yields among seven winter wheat cultivars.

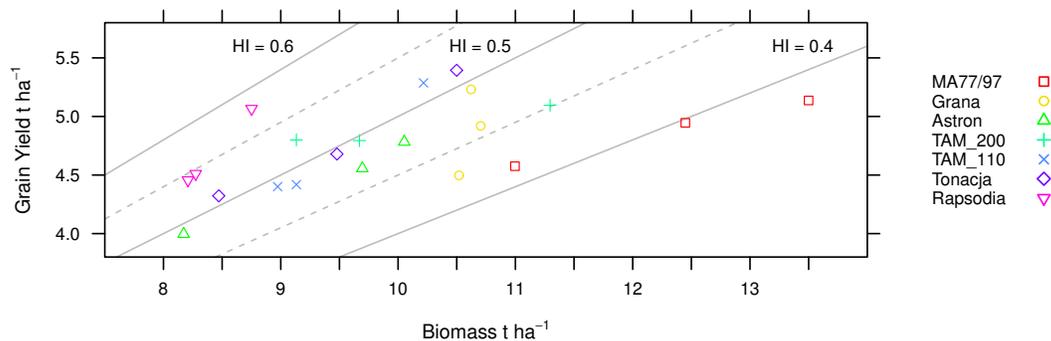


Figure 3. MM-Contour plot for harvest index (HI), grain and biomass yields among seven winter wheat cultivars. Isometric scale was used.

ANALYSIS FOR 32 GENOTYPES

The proposed MM-Contour plot can provide much deeper information about the relationships among grain yield, harvest index and biomass yield than the other presented types of graphs. But with a larger number of genotypes even this type of plot will fail because of too much clutter in the plotting region. This can be seen in Figure 4, which presents the MM-Contour plot for all 32 genotypes studied in the experiment. To improve efficiency of this graph, we propose to use a trellis display. This graphical technique (which can be used for most types of graphs) is simple but very efficient when there are too many groups to present in one graph (here, 32 genotypes constitute 32 groups). The effectiveness of

trellis display is mainly based on its specific construction: data are divided into panels set up in columns and rows. Each panel shows the same variables x and y for variable z, and all panels have a common scale. Cleveland and McGill (1984) and Cleveland (1985, pp. 254) proved this comparing data on a common scale is the most efficient in terms of perception. Becker et al. (1996) confirmed the usefulness of trellis display for the presentation of large data sets. Utilized in various applications, trellis display has also been proposed for agricultural sciences (Ćobanović et al., 2007; Szabó et al., 2008; Kozak et al., 2010; Luo and Kathuria, 2013).

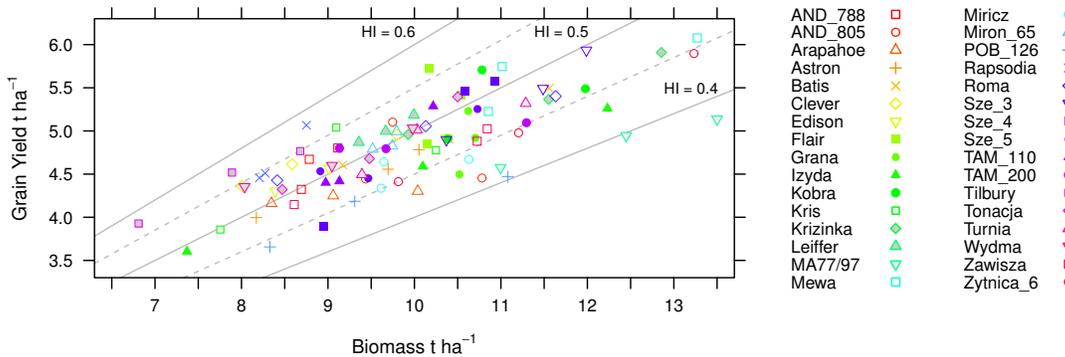


Figure 4. MM-Contour plot for harvest index (HI), grain and biomass yields among 32 winter wheat cultivars. Isometric scale was used.

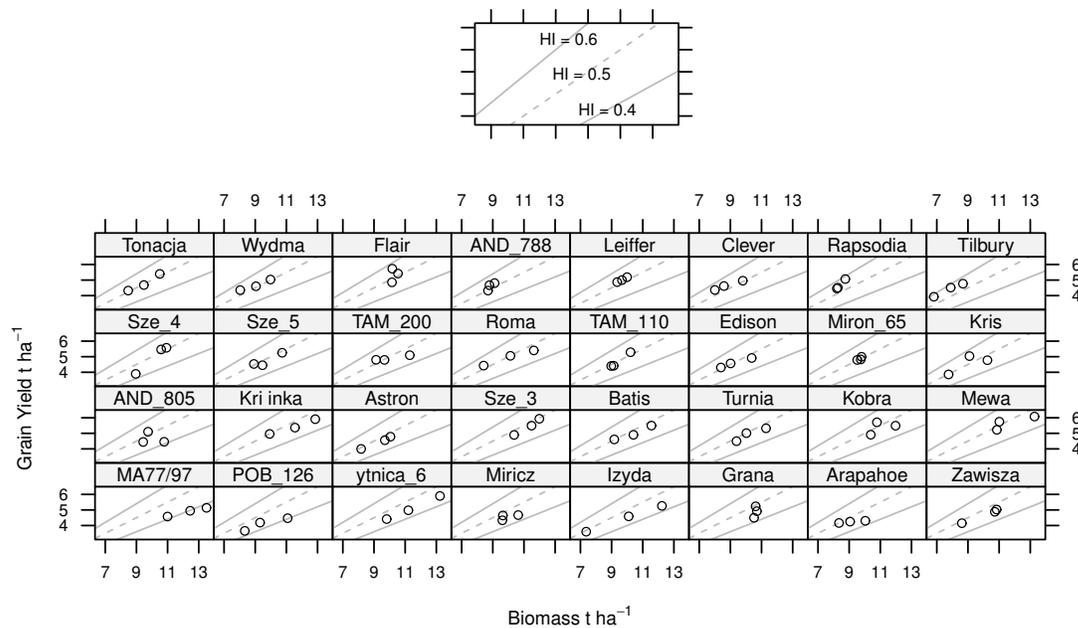


Figure 5. Trellis display of MM-Contour plot for 32 winter wheat genotypes. The genotypes are ordered by increasing harvest index. Isometric scale was used.

Trellis display of MM-Contour plot for the 32 genotypes studied in the present experiment is presented in Figure 5. As was in Figures 3 and 4, biomass yield is presented on the horizontal axis, and grain yield on the vertical, and limits for HI are presented with lines superimposed in each panel. Genotype is the grouping variable so that each genotype has its own panel and thereby its own MM-Contour plot. The panels are ordered according to an

increasing mean value of HI. The trellis display allows for effective analysis and comparison of a relatively large number of genotypes, which would not be possible with any other graph presented in this article. Note that we do not need to use different symbols or color for points representing various genotypes (as we had to in Figures 1-4).

In Figure 5 the genotype with the highest mean HI is presented in the top-right corner, and that with the lowest mean HI in the bottom-left corner; genotypes with increasing mean HI are to the right in a row and higher in a column. Such ordering facilitates genotype-to-genotype comparison with the focus on HI. We can immediately see that the highest HI has Tilbury genotype, which is also characterized by relatively low variability of grain and biomass yields. With this type of graph we can see genotypes with high variability of biomass yield, that is Roma, Krizinka, Izyda, MA 77/67 and Zytница 6, and the lowest variability of biomass yield, that is, Miron_65, Grana and Flair. We could order the graph not only by mean HI, but also mean grain yield, mean biomass yield, or alphabetically, depending on our needs.

DISCUSSION AND CONCLUSION

The visualization technique we propose in this paper consists of several different plots. Stripcharts offer the least information, but in the very easily accessible way. Scatterplot matrix offers some deeper information, but requires much more attention. MM-Contour plot offers even more information that is specific for the multiplicative model (2), and all of that in one single plot, so in the most concise form of the three plots discussed. It requires even more attention than scatterplot matrix, but the information it provides pays off. For a large number of genotypes, trellis version of MM-Contour plot proves its usefulness. It allows one to analyse and present the relationships among the traits that follow a multiplicative model, while the other types of graphs fail to reach this aim. Therefore, when there are too many genotypes to be presented in the graphs we describe, the trellis display for the MM-Contour plot seems a good choice. On the contrary, it does not make much sense to use the trellis display for stripcharts and scatterplots as in Figures 1 and 2, with each genotype constituting a separate panel. One might use the trellis display in which several cultivars are presented in one panel, but this technique would require additional efforts and would not be as effective as the trellis display in Figure 5.

We have presented the visualization technique for the sets of 7 and 32 cultivars from a one-year experiment with three replications. There is, however, no problem in applying the technique for just one cultivar studied across several environments (year or locations or the combinations of both) or for many cultivars grown in many environments. In the latter situation one would have to construct more plots, for example a set of the three plots for each cultivar (so different plotting symbols would correspond to different environments) or, alternatively, for each cultivar (so different plotting symbols would correspond to different cultivars, as was in the above example). In addition, in the case of many replicates for cultivars (or environments), each cultivar (environment) may have its own plot so that the within-cultivar (environment) variation can be distinguished from the between-cultivar (environment) variation.

In this paper, we presented a few types of graphs. Each of them offers a different kind of information about the data or the multiplicative model. In our opinion, all of these visualization techniques should be used together in interpreting the relationships among HI, biomass yield and grain yield. One should start off from the smallest to the greatest difficulty, that is, from stripcharts through scatterplot matrix up to MM-Contour plot and its trellis version. However, once the interpretation is done, it may be sufficient to use and present just MM-Contour plot (and its trellis version). This is mainly due to its very concise form and the large amount of information it conveys. It is worth noting that the visualization technique proposed could be applied for various traits that follow a model similar to that

presented in the equation (2). Examples include mean final yield (which might be yield of grain, seed, root, oil or any other) per an individual plant and/or a number of plants per unit area as components of yield per unit area of most plant species (Hůhn, 1987), and N supply and N uptake efficiency as the components of N uptake (Kozak et al., 2007; Samborski et al., 2008); for other examples refer to Kozak and Verma (2009).

Finally, it is worth noting that the contrary to other known methods of yield component analysis (Fraser and Eaton, 1983; Kozak and Mađdry, 2006), our visualization proposal is not limited to studying the influence of component traits on the final complex trait (grain yield in our example). It rather offers insights into the relationships among the traits, showing what these relationships look like. We do not mean that visualization should replace formal statistical analysis (although in many situations this might be a sensible solution), but we do mean that visualization can be much helpful in interpreting the associations. Therefore, these two types of interpretations—that is, that offered by yield component analysis and that offered by the visualization technique proposed in this paper—are different, and can be applied simultaneously.

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