



## Using a fractional factorial design to evaluate the effect of the intensity of agronomic practices on the yield of different winter oilseed rape morphotypes



Krzysztof J. Jankowski<sup>a,\*</sup>, Wojciech S. Budzyński<sup>a</sup>, Dariusz Załuski<sup>b</sup>, Piotr S. Hulanicki<sup>a</sup>, Bogdan Dubis<sup>a</sup>

<sup>a</sup> University of Warmia and Mazury in Olsztyn, Department of Agrotechnology, Agricultural Production Management and Agribusiness, Oczapowskiego 8, 10719 Olsztyn, Poland

<sup>b</sup> University of Warmia and Mazury in Olsztyn, Department of Plant Breeding and Seed Production, Plac Łódzki 3, 10719 Olsztyn, Poland

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### ABSTRACT

Significant progress in plant breeding and molecular genetics contributed to the development of rape-seed (*Brassica napus* L.) genotypes/cultivars whose biomass (mainly seeds) can be used for a variety of purposes. Those advancements are also used to modify rapeseed production technologies. In systems with many production factors, agronomic measures exert direct and indirect (interaction) effects on crops and yield. Multiple production factors can be analyzed simultaneously with the use of fractional factorial  $s^{k-p}$  designs. A field experiment with a  $s^{k-1}$  fractional factorial design, with 5 factors ( $k=5$ ) at 3 levels ( $s=3$ ), was performed at the Agricultural Experiment Station in Bałcyny (north-eastern Poland) in 2008–2011. The study investigated the responses of semi-dwarf (PR45 D03, Avenir) and long-stem (Visby) heterozygous cultivars of winter oilseed rape (WOR) to the main yield-forming factors (seeding date, seeding rate, spring nitrogen fertilization) and yield-protecting factors (fungal disease management in spring). Seeds of WOR cultivars were sown on 12 and 22 August and on 1 September at three seeding rates of 40, 60 and 80 pure live seeds  $m^{-2}$ . Nitrogen fertilizers were applied at the rate of 80, 160, and 240  $kg\ ha^{-1}$ , and the plots were subjected to 0, 1 and 2 fungicide treatments. The highest yield (4.73  $Mg\ ha^{-1}$ ) was reported for the long-stem cultivar Visby, which was 120 and 620  $kg\ ha^{-1}$  higher in comparison with semi-dwarf cultivars Avenir and PR45 D03, respectively. Early sowing (12 August) was well tolerated by the semi-dwarf variety Avenir and the conventional height cultivar Visby. Early sowing led to a 6% decrease in seed yield of the semi-dwarf cultivar PR45 D03. None of the studied varieties had a positive response to sowing at the beginning of September. Delayed sowing generated more negative effects in semi-dwarf cultivars (decrease in seed yield by around 91  $kg\ ha^{-1}\ day^{-1}$ ) than in the long-stem cultivar (59  $kg\ ha^{-1}$ ). The seeding rate of 80 pure live seeds  $m^{-2}$  (considerably higher than recommended for hybrid WOR cultivars) had the most beneficial influence on seed yield in all tested WOR cultivars. Nitrogen (N) fertilization exerted a yield-forming effect up to a rate of 240  $kg\ ha^{-1}$  in both analyzed cultivars of WOR. The semi-dwarf cultivars and the conventional height cultivar of WOR showed identical responses to the applied fungicide treatments. Single fungicide treatment at the stage of petals falling from the main raceme was most effective. Fungicide withdrawal decreased seed yield by as much as 110  $kg\ ha^{-1}$ . The straw yield of semi-dwarf and conventional height cultivars of WOR was comparable because the former was characterized by a stronger branching structure and a higher number of pods. Contrary to expectations, the long-stem cultivar was characterized by a more favorable harvest index than semi-dwarf cultivars.

This study relies on the  $3^{5-1}$  fractional factorial design which is a fast and relatively cheap method of identifying key agricultural factors and their interactions in the process of determining the yield of new cultivars. The results can also optimize decision-making in agriculture and constitute a basis for establishing effective levels of key operations in the production of new morphotypes and genotypes of agricultural crops.

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\* Corresponding author. Fax: +48 895233243.

E-mail address: [krzysztof.jankowski@uwm.edu.pl](mailto:krzysztof.jankowski@uwm.edu.pl) (K.J. Jankowski).

## 1. Introduction

In the history of civilization, the replacement of farm animals with machinery, the introduction of artificial fertilizers and crop protection products, and breeding progress contributed to a significant increase in agricultural output (Dunn, 2012). European agriculture has attained the third of the mentioned stages of development where cultivars make the greatest contribution to production efficiency (Arseniuk and Oleksiak, 2009; Brisson et al., 2010; Fischer and Edmeades, 2010). In the last 20 years, the progress in cereal breeding reached  $50 \text{ ha}^{-1} \text{ year}^{-1}$  (Poland) and  $90\text{--}128 \text{ kg ha}^{-1} \text{ year}^{-1}$  (France, Great Britain) (Arseniuk and Oleksiak, 2009; Brisson et al., 2010). Farmers' ability to make the most of the progress in breeding winter oilseed rape (WOR) (*Brassica napus* L.) cultivars, estimated at  $65\text{--}85 \text{ kg ha}^{-1} \text{ year}^{-1}$  by Arseniuk and Oleksiak (2009), will largely determine the effectiveness (agronomic and economic) of the applied WOR production technologies (Diepenbrock, 2000; Wittkop et al., 2009).

Until the late 1980s, biological progress in rapeseed breeding involved mainly qualitative changes (Eskin, 2013). The reduction and, subsequently, elimination of erucic acid (EA) from the fatty acid profile and the 15- to 16-fold reduction in glucosinolate (GLS) levels in fat-free seed residues marked the beginning of a new era in rapeseed production. (Przybylski, 2011). In the early 1990s, the yield of rapeseed cultivars was significantly improved (Friedt and Snowdon, 2009; Wittkop et al., 2009) by: (i) continued improvement in the yield of hybrid cultivars (Zand and Beckie 2002; Malhi et al., 2007), (ii) development of/increased resistance to biotic and abiotic stress (Waalén et al., 2014), (iii) increased N-uptake capacity (Kessel et al., 2012), and (iv) changes in phenotype (dwarf and semi-dwarf cultivars) to reduce susceptibility to lodging (Frick et al., 1994; Muangprom and Osborn, 2004; Muangprom et al., 2006).

Winter rapeseed cultivars grown in temperate to cool climates have to be characterized by freezing tolerance. Winter survival of plants depends on a commercial variety of adaptive mechanisms, including cold acclimation for survival at sub-zero temperatures and vernalization for delayed flowering (Waalén et al., 2014). In temperate regions, seasonal synchronization of reproduction requires vernalization which prevents floral transition before winter and promotes transition after winter (Zografos and Sung, 2012). Vernalization maintains winter annuals in a cold hardy vegetative state during winter (Waalén et al., 2014). The freezing tolerance of WOR cultivars also influences plant growth before winter, mainly the number of leaves per rosette (Waalén et al., 2013) and the height of the shoot apical meristem (Jankowski and Budzyński, 2007). The number of leaves formed in fall is determined by the number of heat units and the availability of nutrients, moisture and light (Waalén et al., 2013). In fall, the main production factors (not chemical) that influence the growth of WOR plants are seeding date and seeding rate (Velička et al., 2012). Changes in those parameters directly influence the number of heat units and the availability of moisture and light for plants developing in fall.

Yield is influenced by all production factors, but fertilization, in particular N fertilization, has the greatest yield-forming effect in WOR production (Diepenbrock, 2000; Barłóg and Grzebisz, 2004; Rathke et al., 2005, 2006; Ahmad et al., 2011). Nitrogen also strongly influences other components of the agricultural ecosystem by increasing the competitiveness of weeds (Andersson and Milberg, 1998), pests (Veromann et al., 2013) and pathogens (Sochting and Verreet, 2004) relative to WOR stands.

High dynamics area under rapeseed increased (1 million  $\text{ha year}^{-1}$  between 2003 and 2013, FAOSTAT, 2015) can be attributed to the growing significance of rapeseed in food production (Ackman, 1990), feed production (Nosenko et al., 2014),

industry (Jang et al., 2011), energy generation (Jankowski et al., 2015a) and environmental protection (Jankowski et al., 2014; Jankowski et al., 2015b). The importance of chemical protection against pathogens is growing in rapeseed stands whose proportion in the cropping structure has been increasing steadily (West et al., 2001).

In field experiments with 2–3 experimental factors, the responses of new WOR cultivars to various agricultural inputs can differ significantly from the responses noted in recent agricultural practice. Field experiments with a small number of experimental factors do not support evaluations of various interactions which can significantly affect specific traits in multifactorial production systems.

Multiple production factors can be analyzed in experiments with  $s^k$  factorial design, where  $k$  factors are evaluated at  $s$  levels, usually two or three. A significant limitation of full factorial designs is that the number of combinations which have to be tested increases with a rise in the number of experimental factors, in particular when  $s > 2$ . For example, 3 factors at 3 levels ( $3^3$ ) generate 27 experimental units per replication, 4 factors ( $3^4$ ) generate 81 combinations, and 5 factors ( $3^5$ )–243 combinations that need to be tested. For this reason, the number of combinations should be reduced while maintaining the system's ability to detect significant treatment effects. This goal can be achieved with the use of  $3^{k-p}$  fractional factorial designs where  $k$  factors at  $s = 3$  levels are tested based on  $1/3^p$  (where  $p$  is fraction size) of the set of  $3^k$  experimental units. The separation of  $1/3^1$  experimental units from the full  $3^5$  factorial design reduces the number of combinations to 81 and produces the  $3^{5-1}$  factorial design. The appropriate design generators are selected to evaluate the main effects of 5 factors at 3 levels and the effects of all two-factor interactions in  $1/3$  of experimental units in the full  $3^5$  design. Further reduction ( $1/3^2$ ) to the  $3^{5-2}$  design is possible, and it produces 27 combinations per replicate. Generators of  $3^{5-2}$  designs usually limit the extent of evaluations of the main effects of 5 factors at 3 levels.

Fractional factorial designs were introduced to experimental practice by Finney (1943, 1946, 1949) and Kempthorne (1947), and their popularity increased steadily, especially in industrial experiments (Daniel, 1956; Box and Hunter, 1961a,b; Box et al. 1978; Wu and Hamada, 2009). Fractional factorial designs were rarely used in field experiments due to major differences between agricultural and industrial research and the routine use of standard methods in field experiments.

The popularity of fractional factorial designs continues to be low for the following two methodological reasons which should be eliminated already during design. The first is the knowledge about which treatment effects equal zero or are non-significant enough to be eliminated and which effects are important enough to be incorporated in the model. The relevant knowledge supports the use of mathematical equations as generators for developing the appropriate fractional design. The selected equations should support the estimation of major effects and the elimination of the remaining effects which should be classified as experimental error in the model. The above process is deployed to reduce the number of experimental units in fractional designs. In our study, the applied generator was  $a + b + c + d + e = 0 \text{ mod } 3$ . In agricultural experiments, higher-order interactions deliver the least useful Effects, therefore, they can be omitted or confounded (aliased). This brings us to the second problem. Confounding or aliasing means that two or more effects cannot be separated in data analysis data. If effect B cannot be included in a model because it is confounded with effect A which is included in the model, the apparent magnitude of effect A might not be due to A alone. The effect, as estimated, is due to the combined effects of A and B. The observed effect cannot be ascribed solely to A, unless an extrinsic argument exists to indicate that effect B does not exist or is small relative to effect A.

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