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Assessment of genetic divergence in Rapeseeds *Brassica napus* L. and *Brassica campestris* L. crops for exploitation of host plant tolerance to Aphid *Myzus persicae* (Sulzer)

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The impacts of the aphid infestation on yield of Rapeseeds, *Brassica napus* L. and *Brassica campestris* L., genotypes were studied in an experiment carried out in natural conditions. The attack of aphid on crop and its effects on seed weight were determined using a random plants sampling and by analysis of grain yield. On the basis of pest damage, no genotypes were totally free from aphid *Myzus persicae* (Sulzer) invasion. Distinct genotypes differing in pest tolerance and grain yield were identified from the experiment. The parameters used quantified that genotypes *viz.,* MM-IV/03-5, MM-III/03-4 and NIFA-Raya were the least infested, presented the highest seed weight and showed more resistance to pest. The Abasin-95, 04k 8/13-2 and NH-975/2-4 genotypes showed more susceptibility to pest and were superfluous infested and produced less grain. Present findings revealed that the resistant genotypes can be utilized in breeding programs for development of Brassica varieties having reasonable aphid tolerance at growing stage to receive maximum seed yield.

Key words: Rapeseed, *Brassica napus, Brassica campestris*, aphids, resistance.

INTRODUCTION

Rapeseeds, *Brassica napus* L. and *Brassica campestris* L., also known as oilseed rapes, are bright yellow flowering members of the family Brassicaceae, cultivated mainly for their extremely nutritious and oil-rich seed sources. Rapeseed varieties represent one of the major sources as an edible oil cooking medium. Due to the gap between domestic availability and actual consumption of edible oils, Pakistan has to import a large amount of edible oils due to a probable demand for vegetable oils. The production constraints faced by rapeseed are diverse in nature; the problems of common nature include non availability of superior seed materials to farmers at the correct time, an inadequate research and lack of extension linkages. The prevalence of biotic stress such as aphids and abiotic stress like frost and high temperature causes severe yield loss in the major crop producing areas. Aphids, also known as plant lice, are small, pear shaped, soft bodied insect pests, which feed on plants, usually on the undersides. Aphids have piercing-sucking mouthparts and cause damage by sucking the plant juices. Aphids excrete large amounts of honeydew which provide an excellent medium for the growth of a black fungus called sooty molds. Besides being unattractive, sooty mold may interfere with photosynthesis and retard plant growth (Sarwar, 2008; 2009). Aphids may infest almost any plant, however, they are more commonly found on *B. napus* and *B. campestris* crops. Aphids *Myzus persicae* (Sulzer) (Homoptera: Aphididae) are commonly found on the stems or undersides of young leaves in small colonies. Aphids

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mostly feed on the new plant growth, their feedings make the leaves curl or crinkle and flower buds may become hardened, causing the flowers to be distorted. Usually aphids are not difficult to control with insecticides, but, plants may become re-infested from adjacent areas throughout the crop season. An initial infestation of aphids is usually localized, but, can spread quickly if allowed to develop unchecked (Sarwar and Saqib, 2010; Sarwar et al., 2011).

The concept of Integrated Pest Management (IPM) of Brassica oilseed crops, stresses the need to use multiple tactics to maintain insect pest abundance and damage below levels of economic significance. The role of plant resistance to insects in IPM has been well defined, and insect-resistant crop varieties either elevate the damage tolerance level of the plants to insect pests or suppress their abundance. Thus, by the use of insect-resistant crop varieties, economical benefits occur because crop yields are saved from losses by insect pests and money is saved due to not applying insecticides that would have been applied to susceptible varieties (Sarwar et al., 2004; Sarwar and Sattar, 2013). Nowadays, the variety selection is very difficult due to the proliferation of newly registered lines.

Variety selection is important for producing a crop that contains desirable performance traits and also quality seed to be an economically competitive marketable product. This publication will identify factors to consider in variety selection. Individual producers must decide which factors are most important to their operation, and match a rapeseed variety to those criteria. Present study will provide essential information that can help to a successful pest management in rapeseed crops to maintain pest abundance below tolerate limit. With recent advancements in crop production, this finding will provide the basics to separate the tolerant genotypes from the susceptible.

MATERIALS AND METHODS

Agronomic practices

This experiment was conducted in the field conditions at Nuclear Institute of Agriculture, Tandojam, Sindh, Pakistan. Thirteen genotypes representing the different range of diversity taken from agro climatic zone of Nuclear Institute of Food and Agriculture (NIFA), Peshawar, Pakistan, were raised. Among the germplasm lines the following entries to develop as potentially tolerant genotypes were selected for trial:- 04k 8/13-2, 04k 8/13-18-1, 04k 8/13-12, 04k 9/13-9-2, 04k 9/13-3-4, 04k 12/13-10-1, 04k 12/13-15-2, NH-975/2-4, Abasin-95, Durr-e-NIFA, MM-III/03-4, MM-IV/03-5 and NIFA-Raya. For uniform germination the seedbed prepared were firm, moist and uniform to ensure adequate seed to soil contact, the correct and uniform planting depth and quick moisture absorption, ultimately leading to even germination. The trial was sown in November during winter and planted with a drill after its calibration and settings, to provide accurate seed placement across soil. All other cultural practices were kept normal and uniform for all the experimental units. The experiment was laid out in a randomized complete block design with three replications, and each of the experimental unit measured 1.8 m^2 areas. The genotypes were randomized in each replication in rows 30 cm apart in a well prepared seed bed. The crop was fertilized at the rate of 60-40-40 Kg Nitrogen-Phosphorus-Potassium per hectare in two installments that is, ½ N, the whole P and K at sowing, and remaining ½ N applied at the time of first irrigation. A total of three irrigations were given to grow the crop. For aphid performance data of individual genotypes, the following criteria were considered for evaluating individual lines to obtain information on resistance:-

1. Population dynamics of aphids was studied by counting of aphids from all upper, middle and lower leaves of five different plants, selected randomly in each replicate on fortnightly basis.
2. Estimated the crop yield recorded per 1.8 m^2 of experimental area after harvesting.

Pest population

The core of present efforts was monitoring field grown crop by the proper use of pest scouting. Experimental field was monitored for pests using 10X hand lens to help identify insect pests involved. While sampling, selected 5 random plants per replicate whether infested or not infested with pest in a pattern that covered the entire field site. The aphids were looked under young upper leaves, petioles, and stem during vegetative growth, and during later vegetative stages new branches in the lower canopy were also checked. Within reproductive stages, aphids were also checked on undersides of mid-canopy leaves and stems locations. Each selected plant was sampled separately in a replicate to count all the aphids present and recorded the date and pest density on data sheet.

Grain weight

At crop maturity, from each entry, the crop was harvested and data for the grain weight were recorded. The seed weight was recorded in grams and determined as economic yield for each genotype expressed per 1.8 m^2 of experimental area.

Data analysis

Based on the results, the genetic diversity of each genotype for aphid incidence and seed yield characters were computed with Statistix 8.1 analysis software. Means were separated using the least significant difference (LSD) test at probability of P = 0.05. The capability of a genotype to survive under aphid density for growth period and set seed at harvest was regarded as pest tolerance.

RESULTS AND DISCUSSION

Currently, green peach aphid *Myzus persicae,* was the most common aphid known to infest, debilitate and under some conditions, killing plants. Aphid was usually found in colonies on the underside of leaves. By their piercing-sucking mouthparts, the aphids fed by sucking plant juices and when substantial damage occurred, the plants took on an unthrifty appearance, wilted or changed colors compared to healthy plants and their populations were frequently very spotty. The attacked plants became stunted with poor canopy development and yield impacted before plant symptoms turned out to be readily apparent. During reproductive stages, continuous aphid feeding resulted poor pod and seed setting. Pest scouting

Table 1. Comparative feeding preference and response of various Brassica genotypes to aphid infestation under field conditions.

S/N	Name of genotypes	Aphids population/ plant	Yield in grams (1.80 m^2)
1	04k 8/13-2	68.22[b]	196.70[gh]
2	04k 8/13-18-1	44.66[e]	266.70[de]
3	04k 8/13-12	52.44[d]	233.30[efg]
4	04k 9/13-9-2	32.00[gh]	316.70[c]
5	04k 9/13-3-4	35.33[fg]	283.30[cd]
6	04k 12/13-10-1	44.44[e]	233.30[efg]
7	04k 12/13-15-2	39.33[ef]	253.30[def]
8	NH-975/2-4	60.00[c]	216.70[fgh]
9	Abasin-95	84.44[a]	186.70[h]
10	Durr-e-NIFA	55.55c[d]	230.00[efg]
11	MM-III/03-4	27.55[h]	586.70[b]
12	MM-IV/03-5	20.66[i]	630.00[a]
13	NIFA-RAYA	29.67[gh]	563.30[b]
LSD value		6.18	38.67

Mean values represented by different or same letters are statistically significant or no significant, respectively, at P = 0.05.

efforts for whole season indicated that for the majority of the genotypes, aphid populations were low to high. As far as aphid population is concerned during the growth period when the germplasm material comprising of 13 lines was exposed to natural pest incidence, none of the entries had shown complete relative tolerance for all the test traits. Similarly, grain yield was lesser or more drastically affected under the period of aphid exposure.

Pest population

On the basis of pest population, lines MM-IV/03-5, followed by MM-III/03-4 showed significant tolerance determining 20.66 and 27.55, aphids/ plant, respectively, and were least affected in spite of their exposure to pest. Among other lines observed, entries NIFA-Raya and 04k 9/13-9-2 were at par after pest exposure and took less pest incidence (29.67 and 32.00 aphids/ plant, respectively) as observed on all other genotypes, and were able to stay green and healthy. Thus, on the basis of analysis, such types of genotypes are very useful for mechanized cultivation and to fit the crop in various cropping patterns, especially between the days to first pod matured and days to attain full maturity. Out of other lines, entries Abasin-95, 04k 8/13-2 and NH-975/2-4 were statistically different from all other genotypes and not able to remain green under pest pressure. Nonetheless, these genotypes had shown relatively higher value of aphid density other (84.44, 68.22 and 60.00 per plant, respectively), over all entries (Table 1).

Grain weight

As shown by the production trends and results of experiment, it is apparent that there were greatly significant differences among various rapeseeds genotypes regarding the grain yield. The greatest grain yield (630.00 gm seeds per 1.8 m^2 area) at final harvest was recorded in MM-IV/03-5, which proved significantly more than that of all other genotypes followed by MM-III/03-4 and NIFA-Raya (586.70 and 563.30 gm seeds), which statistically differed from 04k 9/13-9-2 bearing 316.70 gm seeds per 1.8 m^2 area. The genotypes, MM-III/03-4 and NIFA-Raya were statistically at par with one another, and differed significantly from other test materials. The least amount of grain yield (186.70 gm) given by Abasin-95 was statistically different from all other genotypes. Nonetheless, genotypes 04k 8/13-2 and NH-975/2-4 were statistically at par (196.70 and 216.70 gm seeds), but, statistically poorly divergent in grain yield than rest of lines (Table 1).

It is important to note that plant species tested under natural conditions showed different behavior at different growth stages, for instance increased level of aphid population significantly (P = 0.05) reduced the yield parameter. The grain yield decreased with increase in aphid population, however, MM-IV/03-5, proved best of all the genotypes showing proportionally less decrease in grain yield followed by MM-III/03-4, NIFA-Raya and 04k 9/13-9-2 under field conditions. They recovered when exposed to pest and improved their grain weight to the optimum level. In rest of the genotypes, grain yield was drastically reduced and effects of pest density were most pronounced on sensitive genotypes. The Abasin-95, 04k 8/13-2 and NH-975/2-4 genotypes showed more susceptibility to pest and were more infested and produced less grain. They were not able to recover and hence had shown very poor performance. The differentiation in yield parameter within various genotypes might be due to varied genetic constitution of each germplasm lines as well as aphid intensity.

As can be seen from the observed high or low proportion of aphid population and grain yield in *B. napus* and *B. campestris* germplasms, that there was profound genetic heterogeneity at inter-species and intra-species level. Along with genetic diversity, heterogeneity of constituents of essential oils derived from them can be the basis of resistance. As also previously noted, the inconsistency between the molecular and chemotypic diversity observed among the accessions of lemongrass species suggested that genotype and environment interactions also led the diversification of chemical constituents, other than genotypic differences (Sangwan et al., 2001). Several other factors may also constitute resistance in rapes; these may include avoidance or antixenosis, antibiosis and agronomic tolerance. For such crops, avoidance may not be sufficient since its efficacy can be compromised by multiplicity of crops and insects may have to choose even the less preferred host for feeding or oviposition. Antibiosis does provide excellent resistance, but, at the same time exercises maximum pressure on insect. An ideal resistance is a combination of all three mechanisms with tolerance showing least pressure on the insect to adapt (Smith, 1989).

The studies conducted with Brassica collections revealed occurrence of resistance in many accessions. The results of the pest feeding preference/ choice revealed that the aphids showed maximum preference for feeding on Abasin-95, 04k 8/13-2, NH-975/2-4 and Durr-e-NIFA, while, the least preference was shown for MM-IV/03-5 followed by MM-III/03-4, NIFA-Raya and 04k 9/13-9-2. It could be accomplished that the high levels of genetically primed of both antixenosis and antibiosis against aphid may be involved for tolerance in resistant entries. As previously noted by other researchers, the analysis of biochemical data using linear regression revealed that high concentration of lectins was the major contributory factor towards aphid resistance. This conclusion was reinforced by occurrence of high levels of lectin in the resistant *B. fruticulosa* genotypes in those studies. Cole (1994) had also reported high levels of chitin binding lectins in *B. fruticulosa* to be associated with resistance to cabbage aphid, *Brevicoryne brassicae*. The biological activity of lectins against a number of sap sucking and foliage feeding insects had also been well documented (Murdock et al., 1990; Jenson et al., 2002).

Glucosinolates are sulfur-rich plant metabolites of the order Brassicales that function in the defense of plants against pests and pathogens. They are also important in human society as flavor components, cancer-prevention agents, and crop biofumigants. Given sufficient sulfate, many crops are capable of biosynthesizing more glucosinolates than normally occur under typical agricultural growing conditions. The increased glucosinolate content may aid in defense against pathogens and herbivores, but, this varies with the enemy encountered (Falk et al., 2007). Glucosinolates, compounds found in Brassica plants, are toxic to some soil-borne plant pathogens because of the toxicity of their hydrolysis products, isothiocyanates. Other phytochemicals found in Brassica plants, such as phenols and ascorbic acid, may compliment the activity of glucosinolates (Antonious et al., 2009). Bio-chemical analysis of constituent revealed that higher amount of total phenols; surface wax and glucosinolates were responsible for the aphid resistance (Arvind et al., 2009). From the evidence reviewed in this section, it can be argued that Brassica accessions that demonstrated relative tolerance, generally may had contained the highest glucosinolates content in comparison to accessions that had the lowest concentration.

Conclusion

Present varietals evaluation work has shown that there is a lot of variations in yields and rate of pest incidence within different lines of the crop. This indicates a potential for development of high yielding lines from the tolerant varieties that are both pest resistant and high yielding evenly. To achieve this, it will be necessary to cross MM-IV/03-5, MM-III/03-4, NIFA-Raya and 04k 9/13-9-2 genotypes with the promising and early maturing rapeseeds genotypes to reduce the pest abundance and maturity period, and to enhance yield. Further, the non-shattering characteristics in some of the imported varieties should also be incorporated in the local varieties to reduce harvesting losses. The genetic diversity scenario presented herein for the Brassica species and variants would not only be valuable for conservation of their germplasm, but, also useful for breeding new or novel varieties.

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