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Evaluation the potentiality of some parameters to probe the elevated levels of resistance in *Brassica* sp at the early period of aphid infestation in open field condition

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Abstract
The present study aimed to identify parameters for probing the elevated levels of resistance among the *Brassica* genotypes in the field condition at the early time of aphid infestation. Four *Brassica* genotypes (abbreviated as B2, B3, B3 and B12) having elevated levels of resistance (B2≤B3<B4<B12) were considered. In the field condition, differential aphid inoculum, unsynchronized timing of aphid infestation, movement of aphid within field, and no competition of space & food in the host plant made elusive to probe the elevated levels of resistance. A few parameters can probe the elevated levels of resistance in open field condition at the early period of aphid infestation. These parameters are 'Comparison of water content in aphid itself' collected from genotypes having elevated levels of resistance, 'Comparison of water content between aphid probed twig and aphid unprobed twig', and 'Comparison of water content in aphid probed twig and aphid itself' could potentially differentiate the elevated levels of resistance at the early time point of aphid infestation. The present study identified few novel parameters that could be potentially explored in *Brassica*-Aphid interaction biology from the early time of infestation to probe the resistance level in temporal scale.

Keywords: Aphid, *Brassica*, Resistance, Parameters, Field condition, *Brassica*- Aphid Interaction Biology.

Introduction
The essentiality of oil and oil seed by-products in our daily diet were well realized. Biochemically, edible mustard oil is an excellent source of energy in comparison to carbohydrates and proteins. Mustard oil is extensively used in the delicious food preparation, enhancing food palatability and flavor of the food. Mustard oil is also used as hair oil, lubricants, a folk remedy for arthritis, foot ache, lumbago, and rheumatism (Duke and Wain 1981) [5]. The oil seed cake is also used as cattle feed and fertilizers (Reed, 1976) [17]. Mustard seed is also used as a spice in many of the Indian dishes. Young tender leaves of mustard are also popular for salads, garnishing and even as vegetables. In ever growing population throughout the world, the demand for edible oils as well as food are also increasing. Thus, the cultivation of oilseed crops is gaining more importance day by day. In the last few decades, several mustard varieties were released to enhance the productivity. But the major limitation in mustard production is the aphid infestation because this crop is highly vulnerable to mustard aphid (*Lipaphis erysimi*) attack which is the major limiting factor for production (Patel et. al, 2004). The yield loss due to aphid infestation was recorded from 35.4 to 91.3% (Singh and Sachan, 1994) but the loss may be as high as 97% (Yadava & Singh, 1999) [27]. Nearly 250 aphid species out of ~4300 were identified as agriculturally 'pests' (Blackman and Eastop, 2000) [1] that cause economic damage to crop yield. Direct damage was realized due to the huge volume of phloem sap loss resulting in imbalance in the source-sink relationship. The aphid infestation site acts as induced and strong sink resulting in nutrient flow from primary growth zone to the aphid infested zone (Girousse *et al*., 2005) [7]. Further indirect loss of both plant productivity and quality were realized by virus vectoring and honey dew secretion which promotes fungal contamination (Matthews, 1991; Dixon, 1998) [11, 4].
Aphids can proliferate in large numbers within a very short period. Aphids are exclusively phloem feeders (Blackman and Eastop, 2000) [1] and can reproduce clonally by giving live young birth unlike most insects. Moreover, an aphid's embryonic development begins before

its mother's birth. Thus, certain aphids' nymph can mature within a very short period as five days (Dixon, 1987) [3]. These features shorten the generation time that promotes to reach the economic threshold population very quickly in the favorable environmental condition. In the higher plants (including monocot and dicots), the vascular system that is composed of 'xylem' and 'phloem' were evolved to assign as resource and signaling molecule distribution. Characteristically, phloem sap contains the simple sugars, proteins and amino acids and makes the phloem sap very attractive target niche for most of the pathogens and pests. Moreover, the phloem sap is maintained under high pressure, and have high C: N ratio, high osmolality due to elevated level of sugar content. When ruptured or punctured the phloem structures, plant loose huge volume of sieve element sap. Interestingly, instant effective mechanism was evolved to avoid sieve element sap loss due to rupture or puncture through 'occlusion' mechanism. In the susceptible interaction, aphids can tap the sieve element sap efficiently and continuously from phloem tissue (Blackman and Eastop, 2000; Walling, 2000) [1, 23] reversing the occlusion mechanism. The highest compatibility is achieved with the susceptible host plant resulting in intimate and prolong association with the aphid. During this period, aphids can tap the voluminous amount of phloem sap from the host plant. It will be relevant issue to identify the parameters which will be well reflection from the well feeding from the susceptible host plant as compared to restricted feeding realized in the resistance host plant. The ultimate objective of a pathogen is to assure the nutrient availability from the host plant through temporal molecular battle in the susceptible host.

In field condition, response of elevated levels of resistance at the early time of aphid infestation is the challenging job for studying the plant-aphid interaction biology. In the open field condition, differential aphid inoculum to the host plant, unsynchronized timing of aphid infestation, movement of aphid within field, and no competition of space & food in the host plant made elusive to probe the differentiate the elevated levels of resistance. Several parameters identified in the present study are unable to probe the elevated levels of resistance in open field condition at the early period of aphid infestation. For proper probing the elevated levels of resistance at the field condition, genotypes of different known resistant categories were considered to correlate with both plant specific and aphid specific parameters to address the elevated levels of resistance. The present study reported three novel and potent parameters like 'Comparison of water content in aphid itself' collected from genotypes having elevated levels of resistance, 'Comparison of water content between aphid probed twig and aphid unprobed twig', and 'Comparison of water content in aphid probed twig and aphid itself' that could potentially probe the elevated levels of resistance in *Brassica* genotypes at the early period of aphid infestation in open field condition. These novel parameters could potentially be explored in *Brassica*-Aphid interaction biology to properly address the resistance biology as an outcome from molecular battle in the temporal and spacial manner.

Material and Method

Experimental location, and Climate information

All the experiments were carried out in the Instructional farm of Uttar Banga Krishi Viswavidyalaya Pundibari, Cooch Behar, West Bengal, India during the year of 2016-2017. The Terai zone is situated between $21^0$31'N to $27^0$14'N latitude and $86^0$35' E to $89^0$53' E longitude. The farm is specifically located at 26°29'N latitude and 89°76'E longitude and at an altitude of 47 m (154 foot) above the Mean Sea Level (MSL). During the experimental period, the weather reports were presented in Supplementary Table 1.A.

Table 1A: The calculated weather report during growing period of *Brassica sp*.

Year- 2016-17								
Month	Max-Temp (Mean±sd)	Min-Temp (Mean±sd)	Max-RH (Mean±sd)	Min-RH (Mean±sd)	Rainfall (Mean±sd)	Bright Sunshine in Hour (Mean±sd)	Evapotranspiration (EVP) (Mean±sd)	Wind Speed (Mean±sd)
Dec-2016	28.31±0.21	12.31±0.28	80.32±2.12	69.97±1.98	0±0	-	-	-
Jan-2017	26.28±0.35	9.66±0.34	97±0.34	46.84±1.30	0±0	5.15±0.34	1.52±0.09	1.02±0.90
Feb-2017	27.71±0.38	12.11±0.34	96.86±0.66	49.25±1.95	0±0	4.09±0.47	1.99±0.13	1.41±0.09
March-2017	28.41±0.39	15.30±0.52	92.74±1.19	53.06±2.39	2.18±1.25	4.65±0.50	2.67±0.15	2.16±0.17

Soils Characteristics

The soil is characterized as sandy loam in texture, acidic in reaction (pH 4.0-6.8), high in raw humus content, low in water retention capacity, medium to high in total nitrogen content with a low rate of nitrogen mineralization, medium to high in phosphorus status, low to medium in potash content (Mukhopadhyay *et al*., 2008) [13].

Planting Information and Sample Collection

All the mustard plants were maintained in the line sowing. The plant to plant distance was 10 cm whereas the row to row distance was 50 cm. The length of each row was 3 m. Sowing of mustard was completed on 21st December 2016 and seed was harvested on 15th April 2017. Six replications were maintained per genotype in the experimental plot by making six small blocks. Within the block, one replication per each genotype was followed. In brief, 4 genotypes were sown in 6 blocks, and one line for each genotype was maintained in each block. So, total 4 lines for 4 genotypes within a block, and total 24 lines were maintained in the experimental plot. The same experimental plot was also maintained with aphid control by spraying insecticide when required. Regular visits and collection of plant samples at the time of aphid infestation were followed.

Insect species and Plant material

The aphid infested the mustard plant was known as *Lipaphis erysimi* (Kalt.) and the major insect for the Brassica cultivation. The list of the *Brassica sp*. under study are documented in Supplementary Table 1.B.

Table 1B: The genotypes having elevated levels of resistance are listed

Code	Scientific name/Common name	Collected from	Characteristic feature(s)	
B2	*Brassica compresstris*, yellow serson (known as B-9 variety)	Pulses and Oilseeds Research Station, Berhampore, Murshidabad, WB	Susceptible	
B3	BSH-1, *Brassica compresstris*, brown serson	Same as above	Susceptible	Kumar and Sangha, VEGETOS, Vol. 26 (2) : 387-395 (2013) DOI: 10.5958/j.2229-4473.26.2.103
B4	*Brassica juncia*, T-6342	Same as above	Resistant to aphid	Jain and Bhargava, Entomology: Novel Approaches, New India Publishing, 04-Jan-2007 - Science - 550 pages.
B12	*Crambe abyssinica*	GB Pant University of Agriculture and Technology (GBPUAT), Pantnagar, Uttarakhand	Highly resistance to aphid	

Total number of aphid per plant

Aphid generally infest the twig part of plant in *Brassica* initially. For this reason, 10 cm twig length was considered from the tip in all the genotypes for both aphid infested and uninfested condition. The aphid infested twig was cut very gently and taken in paper packets and brought to the laboratory. The counting and separation of the aphids were done with the help of fine painting brush.

Ratio of aphid stage I-IV/V per 10 cm twig

The largest aphid size (5th instar) were counted separately and the rest of the instars (Stage I to IV) were also calculated from the total number of insects. From the counted aphid number, this parameter was calculated.

Per aphid average fresh weight

The total aphid number per twig was measured. Then weight of total aphids was taken with the help of electrical weighing balance in milligram (mg). Per aphid average fresh weight was calculated.

Per aphid average dry weight

After taking the fresh weight of total aphid from a single twig, the aphid samples were incubated in the hot air oven at 50°C for @5 hours per day for five days. These dried samples were considered for measuring weights in the High Precision Electrical Weighing Balance (mg). Per insect dry weight was further calculated by dividing the total dry weight by total number of dried aphids.

Diameter at 5 cm from the tip of twig in mm

Hossain (2017) reported that the aphid resistance was corelated with the host plant resistance in *Dolichos lablab*. For this reason, this parameter was considered. Diameter at the 5-cm position from the tip of the twig was measured with the help of measuring device known as Digital calipers. Digimatic Caliper (Mitutoyo Corporation) was used for measuring the diameter of the plant vine in absolute mode i.e. in mm.

Water content in largest sized leaf and 4th leaf

The highest sized leaf was collected to corelate the elevated levels of resistance among the genotypes. In severe aphid infestation condition, the aphid colonized under the surface of large leaf. For this reason, moisture content in the largest sized leaf was taken as consideration. One leaf per plant was considered. Firstly, the leaf was detached from the plant with the help of scissor and then it was placed air tightly in to a polythene bag for avoiding the loss of moisture through evapotranspiration. The collected sample was then weighed in electrical weighing machine, and its fresh weight was recorded. There after these samples were placed in the hot air oven at 50°C for @5 hours per day for five days. The dry weight of the leaf was then taken in the weighing machine again. The available moisture content was calculated accordingly. The length and breadth of the leaf was also documented. The water content of 4th leaf was measured accordingly stated above in this section.

Water content in aphid unprobed twig

The aphid unprobed twig was also considered as a parameter for probing the elevated level of resistance among the genotypes. The 10 cm twig length from the tip of the vine was cut from the plant and placed into air tight polythene bag to avoid the loss of the leaf moisture due to Evapo-Transpiration (ET) during field to the laboratory movement. After taking the total weigth, these samples were placed in the hot air oven at 50°C for @5 hours per day for five days. After documenting the dried weight, the available moisture content was calculated accordingly.

Water content in aphid

Total aphid from infested twig was separated and taken weight in High Precision Balance. After taking weight, aphids were kept in oven at 50°C for @5 hours per day for three days. With the help of dry weight, the water content was calculated accordingly.

Water content in aphid probed twig

After the separation of aphid, the aphid infested twig was considered for fresh weight. Then the aphid infested twig but without aphids was incubated in oven at 50°C for @5 hours per day for three days. The dried twig was further considered for dry weight and water content was calculated accordingly describe in the previous section.

Probe Penetration experiment

Hossain (2017) reported that the host resistance is corelated

with the toughness of the twig in *Dolichos lablab* against the aphid infestation. The probe penetration experiment was considered in the instrument known as Texture analyzer through Stable Micro System using 2 mm stainless pointed needle (Plant code-P/2N). The penetration length was followed for 1 mm. The highest pressure recorded during penetration was considered as positive pressure. The probe will be retracted and the pressure recorded during this process was further considered as negative pressure.

Result and Discussion

Plants are facing constant stress from pests and pathogens. One of the potent pathogen is insects. Nearly half of the one million insect species are dependent on plant for their food (Wu and Baldwin, 2010) [26], including aphids. Aphids are exclusively phloem-feeders and belong to the family *Aphididae* and order *Hemiptera*. Over 4000 aphid species have been documented and a number of these are known to damage plant health (Dixon, 1998) [4]. Aphids are major economic pests that cause yield loss worldwide especially in temperate regions (Blackman and Eastop, 2000) [1]. Aphids infestation causes water stress in host plant, reduced plant growth, wilting, and importantly, aphids are efficient vectors of economically important plant viruses. In the early 1950's, Dr. R.H. Painter, a well renowned entomologist at Kansas State University, introduced three categories of plant resistance mechanisms to aphids: non-preference, antibiosis, and tolerance (Painter, 1951). The term non-preference was further then replaced by 'antixenosis' by Kogan and Ortman (1978) [10]. Antixenosis is a resistance mechanism, in which a plant either fails to serve as a host for the insect pest, or the insects prefer an alternate host plant (Smith, 2005) [19]. Strong antixenosis may result in starvation even when no alternative host is available. In other cases, antixenosis adversely impacts insect behavior, for example its ability to find sieve elements, thus deterring infestation. Antibiosis is the category in which aphid physiology is affected, resulting in adverse impact on the growth, development and/or, reproduction or even in the survival of the insect (Smith, 2005) [19]. Antibiosis may result from chemical and physical defenses of the plant. It could also result from the absence of sufficient nutrients in the plant (Pedigo, 1999; Smith, 2005) [16, 19]. The third resistance category, tolerance, is the ability of the plant to withstand and/or recover from damage caused by the insect at a scale that is comparable to that on a plant without any resistant characteristics (Pedigo, 1999) [19]. In many cases, tolerance is considered as the most durable kind of resistance against insects, because of the reduced selection pressure for new insect biotypes and less deleterious effects on natural enemies (Flinn *et al*., 2001) [6]. Both constitutive (preformed physical and chemical factors) and inducible defenses involving small metabolites and macromolecules contribute to the overall plant resistance to aphids by impacting insect behavior and physiology (Chen, 2008). In the present study, four genotypes were selected having elevated levels of resistance. So, the resistance levels were reported (Supplementary Figure 1.B). The B2 and B3 were reported be susceptible to aphid, B4 was reported to have enhanced resistance and B12 was reported to be tolerant. In this study, attempt was taken to evaluate some parameters to probe the elevated levels of resistance at the early time of aphid infestation in the open field condition.

Aphid infestation caused significant yield loss in B2, B3 and B4 but not in B12 genotypes.

The seed yield per plant was significantly compromised due to aphid infestation realized in 2016-17 (Figure 1). Only *Crambe abyssinica* (B12) did not compromise per plant yield loss even after aphid infestation as compared to control (plant protection measure was considered). It was well reported that aphid secreted effector molecules to manipulate host cell structure and function to enhance the susceptibility (Mondal., 2017) [19]. But in B12, aphid did not able to modulate the host physiology in temporal scale to enhance the susceptibility resulting in no compromise in yield loss.

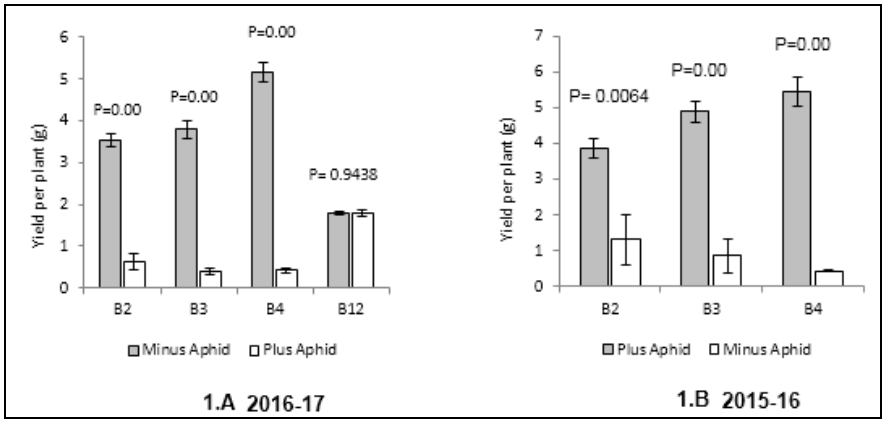
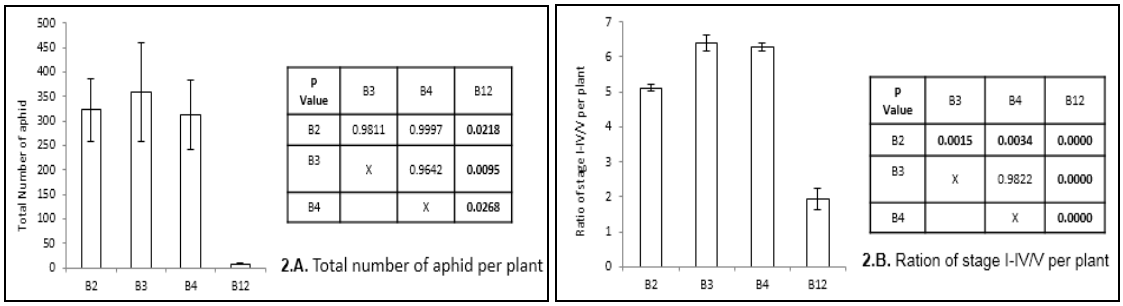


Fig 1: Aphid infestation reduced the yield per plant on B2, B3 and B4 but not in B12 genotypes. Figure 1.A. B2, B3 and B4 showed significant yield loss due to aphid infestation in comparison control where plant protection measure was followed by spraying insecticide. The genotype, *Crambe abyssinica* (B12) showed non-significant yield loss due to aphid infestation in field condition. This genotype, *Crambe abyssinica* (B12) will be categorized as 'tolerance', which allowed certain level of aphid colony establishment without compromising per plant seed yield performance. Figure 1.B. The same trend was reflected in the previous year-2015-16. Aphid infestation reduced the yield per plant on B2, B3 and B4 genotypes maintained in open field condition at Pundibari, Terai zone, UBKV Experimental field

'Total number of aphid per plant' and 'Ratio of stage I-IV/V per plant' did not probe the elevated levels of resistance among the genotypes (B2, B3 and B4) except in B12.

Two common parameters like 'Total number of aphid per plant' and 'Ration of stage I-IV/V per plant' were considered to probe the elevated levels of resistance among the genotypes (B2, B3, B4 and B12) in the open field condition at early time of infestation. In our experimental condition, these two parameters did not able to probe the elevated levels of resistance among the genotypes of B2, B3 and B4 (Figure 2.A and 2.B) but these two parameters could able to differentiate

the enhanced resistance in *Crambe abyssinica* (B12) in field condition at the early time of infestation from the rest of genotypes (B2, B3 and B4). Aphid established the successful penetration in susceptible host plant resulting in more aphid and its progeny. The early time reaction from the host plant was considered as it is the true representative reaction to the aphid infestation. In temporal scale, aphid may modulate host physiology to more susceptible reaction. But in open field condition, initial aphid inoculum and other uncontrolled factors made those two parameters ineffective to probe the elevated levels of resistance among the B2, B3 and B4 except B12.



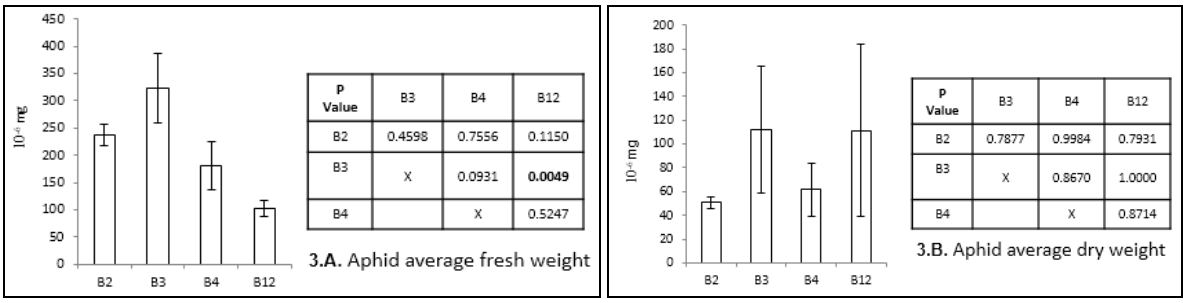
P Value	B3	B4	B12
B2	0.9811	0.9997	**0.0218**
B3	X	0.9642	**0.0095**
B4		X	**0.0268**

P Value	B3	B4	B12
B2	**0.0015**	**0.0034**	**0.0000**
B3	X	0.9822	**0.0000**
B4		X	**0.0000**

Fig 2: Evaluation of the parameters 'Total number of aphid per plant' and 'Ration of stage I-IV/V per plant' for differentiation of the elevated level of resistance among the genotypes. Figure 2.A. Total number of aphid per plant was not able to differentiate the elevated level of resistance among the genotype of B2, B3, and B4. But, the genotype, *Crambe abyssinica* (B12) maintained reduced aphid number per plant in the open field condition at the early time of aphid infestation. The total number of aphid per plant was able to separate *Crambe abyssinica* (B12) from the rest of genotypes. Figure 2B. Parameter 'Ration of Stage I-IV/V per plant was able to separate the enhanced resistance in *Crambe abyssinica* (B12) from rest of genotypes (B2, B3 and B4). These two parameters differentiated the elevated levels of resistance of B12 from the rest of genotypes in both years but not stably differentiate among the genotypes of B2, B3 and B4 in both years, 2016-17 and 2015-16

'Per aphid average fresh weight' and 'Per aphid average dry weight' did not probe the elevated levels of resistance among the genotypes of B2, B3, B4, and B12.

These two parameters could not differentiate the elevated levels of resistance among the genotypes (B2, B3 and B4) (Figure 3.A and 3.B). The aphid collected from these genotypes at the early time of aphid infestation possessed highly variable number of aphid populations within the same genotype and contributed high standard error resulting in non-significant difference.



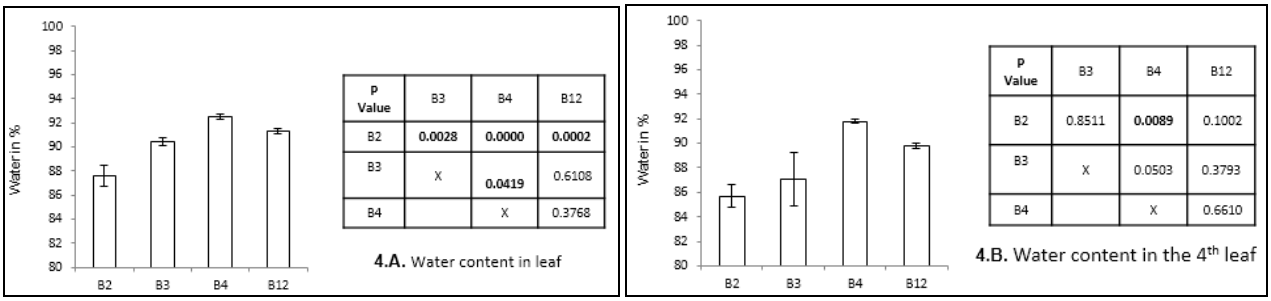
P Value	B3	B4	B12
B2	0.4598	0.7556	0.1150
B3	X	0.0931	**0.0049**
B4		X	0.5247

P Value	B3	B4	B12
B2	0.7877	0.9984	0.7931
B3	X	0.8670	1.0000
B4		X	0.8714

Fig 3: The parameters 'Aphid average fresh weight' and 'Aphid average dry weight' could not differentiate the elevated level of resistance among the genotypes. Figure 3.A Per aphid average fresh weight did not differentiate the elevated level of resistance among the genotypes of B2, B3,and B4. This parameter could able to differentiate the resistance level of B12 from B3 but not from B2 and B4. Figure 3.A. Per aphid average dry weight could not differentiate the elevated level of resistance among the genotypes of B2, B3, B12 and B12

Leaf water percentage did not differentiate the elevated levels of resistance among the genotypes.

These two parameters like 'Water content in largest sized leaf' and 'Water content in 4th leaf' could not potentially differentiate the elevated levels of resistance among the genotypes (B2, B3, B4 and B12) (Figure 4.A and 4.B). The diameter at 5 cm position in the twig was not also correlated with the resistance levels among the genotypes (Figure 5.A).



P Value	B3	B4	B12
B2	**0.0028**	**0.0000**	**0.0002**
B3	X	**0.0419**	0.6108
B4		X	0.3768

P Value	B3	B4	B12
B2	0.8511	**0.0089**	0.1002
B3	X	0.0503	0.3793
B4		X	0.6610

Fig 4: Evaluation of the parameter 'Water content in leaf' to correlate with the elevated levels of resistance among the genotypes of B2, B3, B4 and B12. Figure 4.A. Water content in largest sized leaf. The water content of largest sized leaf in the enhanced resistant genotypes (B4 and B12) were found to be higher than susceptible genotypes (B2 and B3). Figure 4.B. Water content in the 4th leaf. The water content in enhanced resistant genotypes (B4 and B12) were higher than susceptible genotypes (B2 and B3). The moisture content did not positively correlate with aphid resistance

'Water content in aphid unprobed twig' could not potentially differentiate the elevated levels of resistance among the four genotypes.

The above said parameter could not potentially differentiate the elevated levels of resistance among the genotypes (B2, B3, B4 and B12 (Figure 5.B). The genotype having highest resistance had the higher water content in the unprobed twig.

'Water content in aphid itself' could potentially differentiate the elevated levels of resistance among the four genotypes.

The above said parameter could potentially differentiate the elevated levels of resistance among the genotypes (B2, B3, B4 and B12 (Figure 5.C). The main objective of aphid is to assure the continuous tapping of the sieve element sap and keep moister level in aphid body itself for normal physiological functioning. The resistance levels of host plant could potentially be reflected on tapping the sieve element sap from the host plants. In the Figure 5.C, it is interesting finding is that the highest resistant plant, B12 allowed the restricted tapping of the phloem sap ingestion which was reflected in reduced water content in aphid itself. In temporal scale, aphid can manipulate the host cell structure and function. The initial response of genotypes having elevated levels of resistance is the true representation of reaction at the early period of time. Aphid grown in genotype having enhanced resistance showed reduced water content as compared to water content in aphid grown in susceptible hosts like B2 and B3 (Figure 5.C). The aphid water content grown in B4 have lower water content as compared to B12 and higher water content as compared to both B2 and B3 (Figure 5.C). The parameter 'water content' in aphid grown in different genotypes having elevated levels of resistance is found to be correlated with elevated resistance levels. Thus, this parameter may be explored to monitor the resistance mechanism in temporal scale how effector molecules are responsible for degradation the initial enhanced resistance at the early time of aphid infestation.

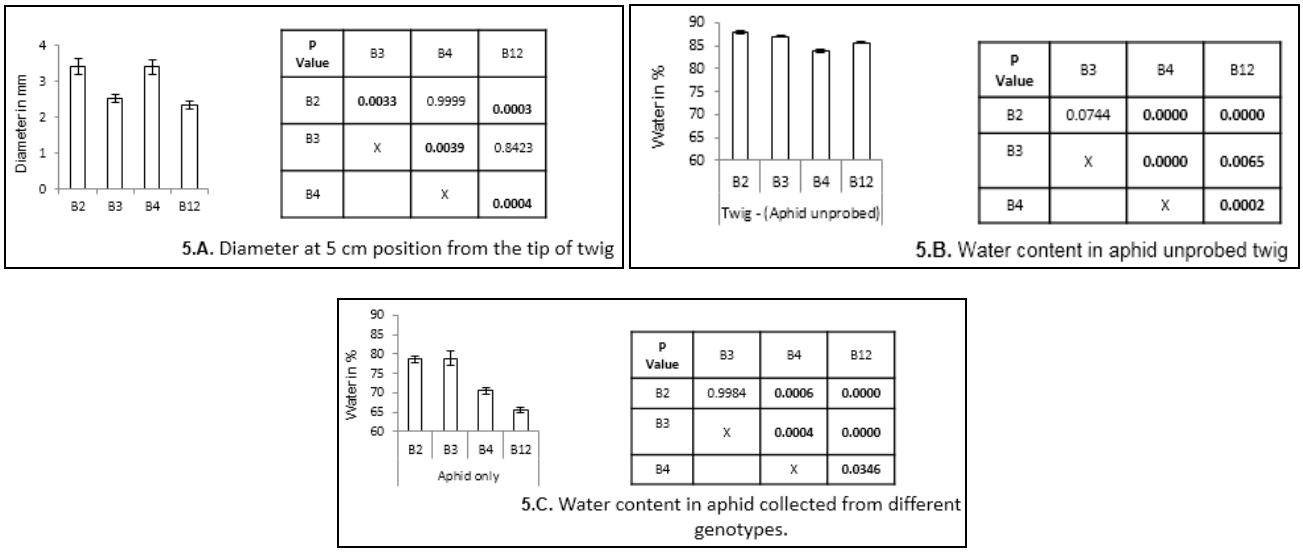


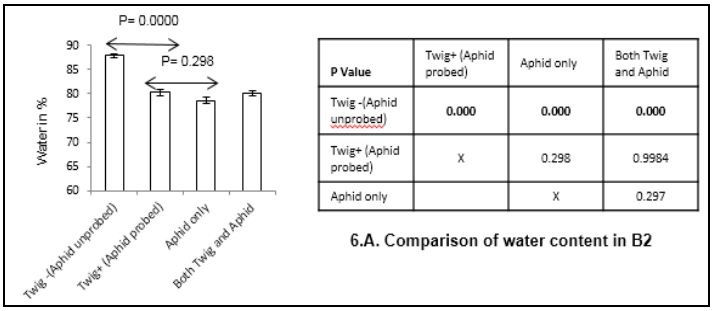
Fig 5: Evaluation of some parameters to correlate with the elevated levels of resistance among the genotypes. 5.A. Diameter at 5 cm position from the tip of twig. The diameter of different genotypes was not correlated with the elevated level of aphid resistance. Figure 5.B. Water content in aphid unprobed twig of B2, B3, B4 and B12. This parameter was able to differentiate the elevated level of resistance among the genotypes but not between B2 and B3. Figure 5.C. Water content in aphid grown in different genotypes. The water content of aphid grown in different genotypes could able to differentiate the elevated level of resistance among the genotypes except between B2 and B3 which belong to the same class of resistance group (susceptible)

'Comparison of water content between aphid probed twig and aphid unprobed twig' and 'Comparison of water content in aphid probed twig and aphid itself' could potentially differentiate the elevated levels of resistance

In the susceptible host, aphids can tap the phloem sap after establishing the successful stylet penetration but in resistant genotype, phloem and outside phloem based resistance limit the sap ingestion from the phloem tissue continuously resulting in less volume of sap ingestion. Considering this background knowledge, parameters are formulated like 'Comparison of water content in aphid probed twig and aphid unprobed twig'. Based on result from different genotypes having elevated levels of resistance, it was found that the significant and non-significant difference in water content in aphid probed twig and aphid un-probed twig among the genotypes having elevated levels of resistance (Figure 6.A, 6.B, 6.C and 6.D). In addition, the 'Comparison of water content in aphid probed twig and aphid itself' was also considered to probe the difference of resistance among the genotypes (Figure 6.A, 6.B, 6.C and 6.D). It was found that these parameters could potentially differentiate the elevated levels of resistance at the early time of aphid infestation in the field condition. Based on result, it was realized that aphid infestation could reduce the water content in the aphid infested twig and indicating overcoming any physiological hardship from host plant resulting in voluminous amount of sap sucking. The close and intimate association with the susceptible host plant equilibrate with the water content in aphid itself with the aphid infested twig. This very interesting finding was also indicative the passive ingestion of sap sucking from the phloem tissue where the sap was maintained by under high pressure. Aphid follows passive ingestion from the phloem of the host plant otherwise the water content of aphid itself will be significantly higher as compared to aphid probed twig if there is any active sucking of phloem sap. It was very interesting finding that aphid infestation could reduce the water content in a significant way as compared to aphid unprobed twig in susceptible host plant (B2 and B3). But in B4 genotype which was reported as having enhanced resistance, the aphid infestation could not able to reduce the

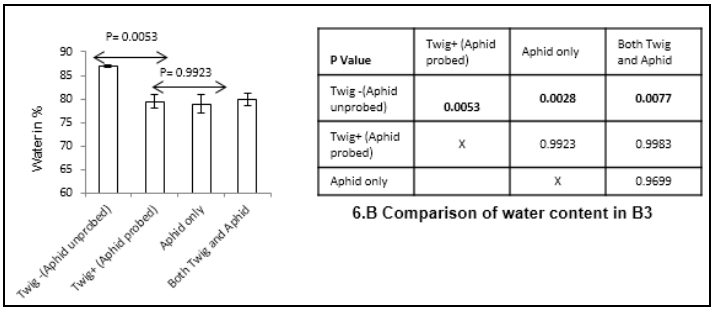
water content in aphid infested twig as compared to aphid uninfested twig (p=0.2027). later on, B4 genotype ultimately compromised the yield loss per plant due to aphid infestation but initially showed enhanced resistance at the early time of aphid infestation. In temporal scale, aphid can manipulate the host cell structure and function for favoring the sap ingestion from the phloem tissue. In B12 genotype, aphid infestation did not reduce the water content in aphid infested twig as compared to aphid uninfested twig (p=0.8355). So, this parameter is potent one to probe the elevated level of resistance among the genotypes having differential level of resistance.

Another parameter, 'the water content of aphid infested twig and aphid itself' was also potent enough to differentiate the resistance levels among the genotypes. In B2 and B3, it was found that the water content of aphid itself was non-significantly maintained the water content of aphid infested twig (p= 0.298 in B2, P= 0.9923 in B3) (Figure 6.A, 6.B,). Bit in genotypes having enhanced level of resistance like B4 and B12, the reverse finding was realized (p=0 in both B4 and B12) (Figure 6.C and 6.D). In these genotypes, aphid was facing hardship to suck the phloem sap resulting in differential water content level with the host twig.



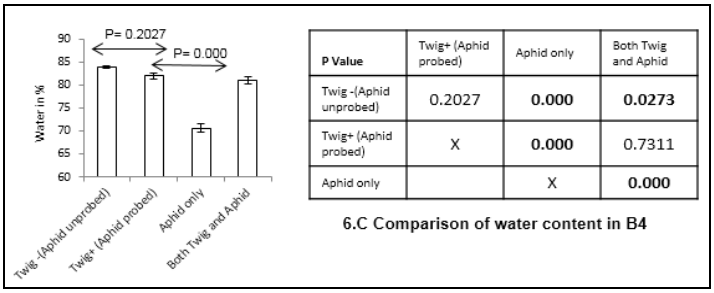
P Value	Twig+ (Aphid probed)	Aphid only	Both Twig and Aphid
Twig -(Aphid unprobed)	**0.000**	**0.000**	**0.000**
Twig+ (Aphid probed)	X	0.298	0.9984
Aphid only		X	0.297

6.A. Comparison of water content in B2



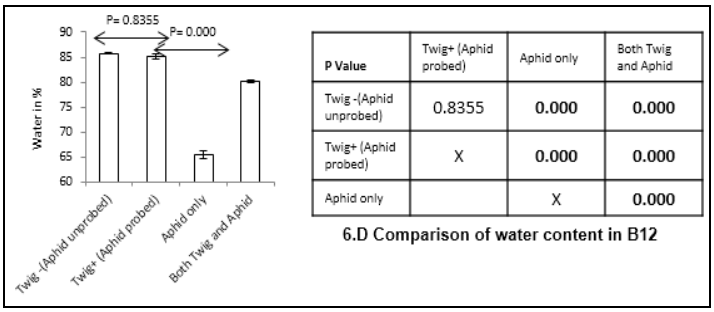
P Value	Twig+ (Aphid probed)	Aphid only	Both Twig and Aphid
Twig -(Aphid unprobed)	**0.0053**	**0.0028**	**0.0077**
Twig+ (Aphid probed)	X	0.9923	0.9983
Aphid only		X	0.9699

6.B Comparison of water content in B3



P Value	Twig+ (Aphid probed)	Aphid only	Both Twig and Aphid
Twig -(Aphid unprobed)	0.2027	**0.000**	**0.0273**
Twig+ (Aphid probed)	X	**0.000**	0.7311
Aphid only		X	**0.000**

6.C Comparison of water content in B4



P Value	Twig+ (Aphid probed)	Aphid only	Both Twig and Aphid
Twig -(Aphid unprobed)	0.8355	**0.000**	**0.000**
Twig+ (Aphid probed)	X	**0.000**	**0.000**
Aphid only		X	**0.000**

6.D Comparison of water content in B12

Fig 6: Evaluation of the parameter 'Pairwise comparison of water content among aphid probed twig, aphid itself and aphid unprobed twig' in all genotypes (B2, B3, B4 and B12). Figure 6.A. Water content of aphid probed twig was significantly lower as compared to aphid unprobed twig (p=0.00) due to aphid infestation in B2 genotype whereas water content of aphid probed twig and aphid itself was comparable (p=0.298). Figure 6.B. Water content of aphid probed twig was significantly lower as compared to aphid unprobed twig (p=0.0053) due to aphid infestation in B3 genotype whereas water content of aphid probed twig and aphid itself was comparable (p=0.9923). Figure 6.C. Water content of aphid probed twig was comparable to aphid unprobed twig (p=0.2027) due to aphid infestation in B4 genotype whereas water content of aphid probed twig was significantly higher as compared to water content of aphid itself (p=0.00). Figure 6.D. Water content of aphid probed twig was comparable to aphid unprobed twig (p=0.8355) due to aphid infestation in B12 genotype whereas water content of aphid probed twig was significantly higher as compared to water content of aphid itself (p=0.00).

Probe Penetration Experiment for mearing pressure required for penetration for 1 mm probe and highest pressure required to retract could not able to differentiate the elevated levels of resistance.

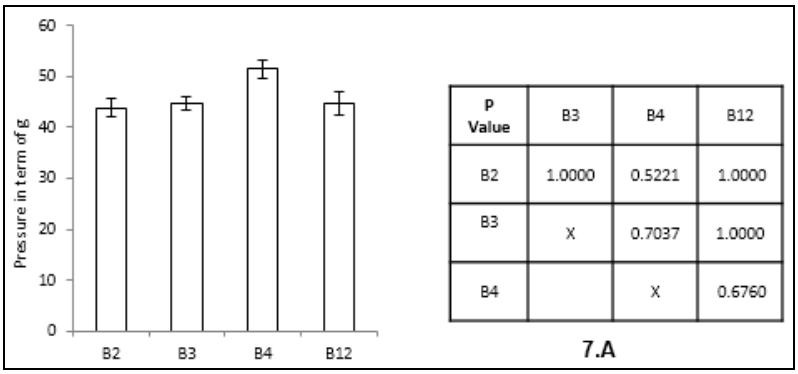
The above two parameters were not able to differentiate the resistance levels of four genotypes (B2, B3, B4 and B12) having elevated levels of resistance (Figure 7.A and 7.B). So, the tightness of tissue at 5 cm position was not correlated with the host resistance against aphid in this genotype.

The compatibility between aphid and its susceptible host plant depends on successful molecular exchange for better access of energy rich nutrient present in sieve element sap. The intimate association with the host plant is achieved in susceptible genotype through successful stylet penetration resulting in enhanced aphid multiplication due to well tapping of voluminous amount of sieve element sap. In resistant host plant, aphid secreted molecules which are recognized by host plant defense system induced effective response and impacts negatively on aphid's survival, growth, and multiplication. The effector molecules play pivotal role in establishing compatibility in the susceptible host plant as well incompatibility with the resistant host plant. Recent findings also solidify the idea that effector molecules play crucial role in successful salivation resulting in sustained contact with the host plant. Due to prolong contact with the sieve element, aphid can tap voluminous amount of phloem sap which is rich in simple sugar and amino acids. The presence of long and slender stylet in aphid makes easy to tap the sieve element sap from phloem tissue. The stylet could reach efficiently into the host sieve element cell to tap nutrient rich sieve element sap. Two canals present in the aphid stylet. One canal is known as saliva canal (SC) that is assigned for saliva secretion into the host tissue. The other canal is for food canal (FD) that functions for tapping the sieve element sap. Characteristically, both FC and SC are merged at the tip of the stylet and make a common duct at the tip portion of the stylet (Uzest *et al*., 2010) [22]. The way of navigation of stylet to the host tissue is predominantly intercellular for minimizing cell damage for averting substantial wound related defense mechanism (Miles, 1999; Walling, 2000; Tjallingii, 2006) [23, 21, 12]. During stylet penetration into the host tissue, aphid secretes two types of saliva known as gel saliva (GS) and watery saliva (WS), respectively. Gel saliva facilitates successful penetration of aphid stylet as well as minimizes the physiological contact with the host plant to avert the plant defense response. Watery saliva is involved in successful salivation due to presence of effector molecules having role in preventing and/or reversing the sieve element occlusion (SEO) (Will *et al*., 2007, 2009). Before initializing the ingestion phase, aphid secretes small amount of watery saliva into sieve element cell to reverse the occlusion mechanism and ensures the accessibility of sieve element sap. If the taste is good and favorable, aphid starts ingestion from the sieve element cell (Tjallingii, 1990, 2006) [20, 21] and continues sap sucking. Aphid explores secreted effector molecules present in saliva from initiation of probing to continuation of feeding. After establishing successful salivation, the plant-aphid interaction is specified to the interaction between aphid's stylet and host's sieve element cell (Mondal., 2017) [19]. The interface between stylet environment and sieve element cell are the site for signal exchange that develops compatibility or incompatibility based on susceptible or resistant host plant. Effector molecules present in the aphid saliva establishes the compatibility when effector molecules can modulate the host cell structure and/or function.

Due to molecular battle in temporal and spacial manner, aphid may establish either compatible association or incompatible association resulting in susceptible or resistant reaction. The main target of any pathogen is to access the nutrient present in host plant for pathogen's survival, growth, and multiplication. Aphid explores the saliva derived effector molecules to establish close association with the susceptible host plant. This close and intimate association impacts on aphid and plant specific parameters that need to be identified at the field condition at the early time of aphid infestation for identifying genotype showing resistance reaction at initial. In the present study, four parameters were recognized to differentiate the elevated levels of resistance among the genotypes of B2 (susceptible), B3 (susceptible), B4 (resistant)and B12 (tolerant) but B2, B3 and B4 showed yield penalty in harvesting. It is interesting that B4 showed initial enhanced resistance at the early period of aphid infestation but showing yield penalty finally whereas B2 and B3 showed initial susceptible reaction as well as showed yield penalty at harvesting. It will be interesting study to tract the resistance breakdown in temporal scale in B4 genotype.

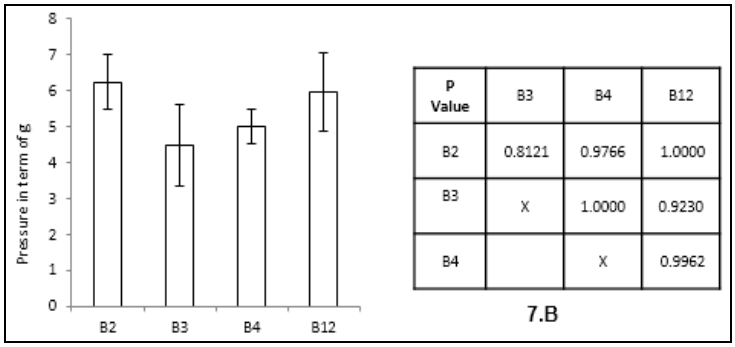
Out of four, one parameter like 'Water content in aphid' could potentially differentiate the elevated levels of resistance among the four genotypes. But this parameter required reference genotypes like susceptible genotype for comparison. Another two parameters like 'Comparison of water content between aphid probed twig and aphid unprobed twig' and 'Comparison of water content in aphid probed twig and aphid itself' could potentially differentiate the elevated levels of resistance. The present study suggests that three parameters are identified as novel parameters like 'Water content in aphid', 'Comparison of water content between aphid probed twig & aphid unprobed twig' and 'Comparison of water content in aphid probed twig & aphid itself' could potentially explored in *Brassica*-Aphid interaction biology in temporal and spacial scale to understand at the molecular dissection in mustard crop.

Based on finding, a model was developed to address the elevated level of resistance a



P Value	B3	B4	B12
B2	1.0000	0.5221	1.0000
B3	X	0.7037	1.0000
B4		X	0.6760

7.A



p Value	B3	B4	B12
B2	0.8121	0.9766	1.0000
B3	X	1.0000	0.9230
B4		X	0.9962

7.B

Fig 7: Probe Penetration Experiment for measuring the tightness of vine. Figure 7.A. Probe Penetration Experiment for measuring positive pressure to penetrate 1 mm at the psotion of 5 cm from the tip of vine. The data showed that the tightness of all genotypes were the same and comparable. Figure 7.B. Probe Penetration Experiment for measuring back pressure to retract 1 mm pointed probe. The data showed that the tissue at 5 cm position have the same tightness and comparable

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