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Aphid colony establishment in *Dolichos lablab* was correlated with the vine diameter variation

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Abstract

Dolichos lablab served as an excellent compatible host for aphid colony establishment in vine/twig region. In the present research, it was observed that aphid colony establishment was significantly higher in higher diameter (HD) vines as compared to lower diameter (LD) vines within the same genotype. In addition to, the HD leaf petiole as well as HD stems also supported significantly higher aphid population establishment as compared to lower diameter (LD). The aphid population establishment within LD vine in different segment of 10 cm from tip having significant vine diameter was not significantly different. Characteristically, within HD vines, the aphid population in different segment of 10 cm from tip having significant vine diameter was significantly different. This characteristic modulation of differential aphid colony establishment in vine, stem, petiole and leaflet's vein within the same genotype was correlated to diameter variation. The HD-specific facilitation promoted aphid population establishment in HD-vine, stem, leaf petiole and leaflet's vein. This information may be the first report that the vine/twig diameter benefited aphid colony establishment.

Keywords: Dolichos lablab, aphid population, diameter, vines, stem, leaf petiole, leaflet's vein

Introduction

One of the characteristic features of the higher-level plants was the presence of the vascular system that was responsible for resource allocation of nutrients and water from shoot to root and root to shoot, respectively. The vascular system was composed of phloem and xylem. The phloem sap was composed of carbohydrate, proteins and amino acids in significant amount. This characteristic feature of phloem sap made a highly favorable target for pathogens and pests (Gündüz and Douglas, 2009)^[5]. Aphids targeted the sieve tubes to consume the nutrient rich sap. For this purpose, aphid explored the most fitted slender 'stylet' for probing the host tissue preferably intercellular way to reach in a very effective way into the phloem vascular system. Prior to sieve-tube feeding, an aphid stylet made its establishment the successful penetration. Once established the stylet contact with sieve element, aphid constantly ingested phloem sap for nutrition and rapid proliferation.

Over the past thirty years, Arabidopsis thaliana had been explored as a model plant to study plant biology covering all aspects of biology like growth, development and adaptation to the environment (Koornneef and Meinke, 2010)^[10]. Arabidopsis had also utilized for dissecting the plant-insect interactions as a model to understand the basic biology and genetics of plant defense response to sucking insect. In the present study, the genotype of *Dolichos lablab* contained variable size of vine diameters. The aphid somehow took this advantage from relatively higher diameter vines. Dolichos lablab belonged to the family Fabaceae where another effective mechanism existed in the sieve elements. The sieve element of Fabaceae contained elongated protein bodies, known as 'forisomes' (Knoblauch et al., 2003)^[8]. It was reported that this protein had ability to switch structural transformations from the crystalloid state to dispersed state i.e., from co-aligned fibrils to a "slime-body" with dispersed fibrils (Palevitz and Newcomb, 1971)^[15]. This transition was very rapid but reversible. This rapid conformational change from shortened longitudinal forisomes to radial expansion increased the several folds volume (Peters et al., 2008) ^[16]. So, for isomes disperse state upon wounding occludde sieve tubes to avoid very costly nutrient rich sieve element sap (Knoblauch and vanBel, 1998).

In the same genotype, a number of both offensive and defense mechanisms exist (Mondal., 2017, 2020; Sengupta *et al.*, 2010; Chakraborti *et al.*, 2009; Louis *et al.*, 2012; Mondal *et al.*, 2012; Mondal., *et al.*, 2018) ^[12, 13, 21, 1, 6, 14]. The type of defense mechanism might be grouped

into physical, chemical, genetical etc. Again, plant showed defensive action against the insects includes both constitutive and preformed factors such as physical barriers (cuticle, trichomes, spines, thorns, etc.) and stored insecticidal compounds. More strikingly, insect infestation also induced physical defenses in plants. For instance, density of trichomes and spines were significantly increased after insect attack in tomato (Solanum lycopersicum) and horsenettle (Solanum carolinense), respectively (Tian et al., 2012; Kariyat et al., 2013) ^[22, 7]. In the present study, only the difference in diameter promoted more aphid number found in HD vines, stem, leaf petiole as well as leaf veins. Significantly higher diameter within different parts of the same HD vine also modulated the aphid colony establishment. This present research communication focused on the characteristically different aphid colony establishment due to diameter variation in different plant parts.

Materials and Methods

Plant material and Planting

The *Dolichos lablab* seed was shown on October 15, 2015. The plant growth was maintained in the farm. The plant canopy was maintained in iron structures of the dimension of 2.4 m x 1.84 m x 0.8 m (Length x Breath x Height).

Insect

The plant was naturally allowed to infest the insect without taking any plant protection measure. During the early infestation time, the insect data was taken.

Age and stage of data collection from the plant

Data was collected after the aphid infestation at the stage of growing colony of 48 days old plant. In the growing aphid colony was considered.

Diameter measurement of the HD and LD vine

Digimatic Caliper (Mitutoyo Corporation) was used for measuring the diameter of the plant vine and other plant parts in millimeter (mm). The selected HD and LD vines were divided into three regions from tip of the vines. The 10 cm length from the vine tip was considered as first segment (HD or LD -1). The second segment was also 10 cm started from the end of first segment and continues up to 10 cm and abbreviated as HD or LD-2. Likewise, the third segment was of 10 cm length started from the end of second segment and continued up to 10 cm. Each segment of vine diameter was taken in the midpoint of each 10 cm vine region. Three diameter data were from each vine was taken and average of three data was considered for vine diameter calculation. Total length of a vine was considered to be 30 cm.

Statistical Analysis

All the statistical calculation was performed by using the Minitab 15 Statistical Software. Comparison between two set of data was followed by General Linear Model.

Results

Dolichos lablab was a very much susceptible to sap sucking insect (aphids) in Indian climatic condition. As a conventional practice, insecticide spray was only solution to reduce the aphid infestation. Already, it was an alarming situation that needed avoidance of the usage of insecticide spraying that already attacked on ecology from the grass root level. It was better to explore the plant resistance mechanism to combat the aphid problem. For achieving the goal, basic knowledge of resistance mechanism in breeding program. In this study, the variety of *Dolichos lablab* was a very much popular in West Bengal but suffers heavy establishment of aphid. But within the same genotype, differential aphid colony establishment was observed.

(HD and LD vines modulated differential aphid colony establishment

Characteristically, it was found that variable diameter of vines was found in a single genotype. This discriminated botanic behavior of supporting the LD and HD vines within the same plant explored by aphid for its colony establishment. It was observed that selected LD and HD vines categories were statistically significant different in diameter (Fig. 1.A; p=0.000). Moreover, it was uniquely found that HD vine supported a significant number of aphids (Fig. 1.B; p=0.007) to the entire vine (total length=30 cm). This botanic behavior of vine diameter was efficiently explored by aphid for its The increased higher colony establishment. aphid proliferation in HD vine must be due to sufficient phloem sap sucking from sieve element. Well feeding promoted the aphid proliferation as compared to LD vine.

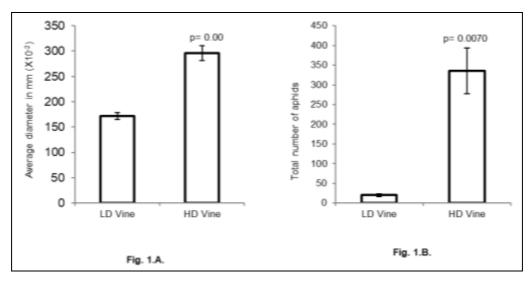


Fig 1: A.B Higher diameter (HD) and lower diameter (LD) vines recorded significantly differential aphid colony establishment.

Fig. 1.A. The vines were grouped based on diameter collected from the same plant, *Dolichos lablab*. The 30 cm vine length from tip was considered. Further, 30 cm vine length was segmented into three region of 10 cm each and started from each vine tip. The data of vine diameter was taken in the midpoint of each 10 cm segment. Three data from a single vine were recorded. The average diameter of three segments in each vine was considered for calculation. The vine diameter was found to be highly significant (p=0.00).

Fig. 1.B. Total numbers of aphids were counted in each 30 cm HD as well as LD vines. The aphid numbers were also significantly higher in HD vines (p = 0.007).

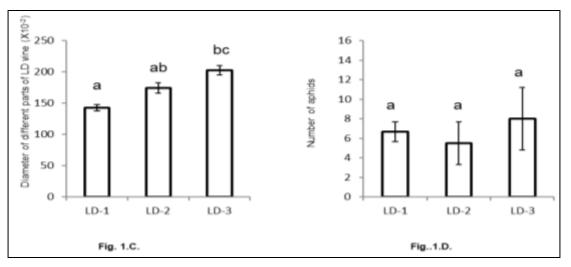


Fig 1: C.D. Aphid colony distribution within different segments in the Lower diameter (LD) vines.

Fig. 1.C. All the vines in LD category were further considered for stem diameter recording. The 30 cm vines were segmented into three regions i.e., LD-1 (1st 10 cm length from tip of the vine), LD-2 (2nd 10 cm length from the end of LD-1), LD-3 (3rd 10 cm length from the end of LD-2). In each region (LD-1, 2 and 3), the vine stem diameter was recorded in the midpoint. Three data from a single vine were considered

separately. The LD-1 vine diameter from all the LD vines were considered for calculation. The vine diameter was found to be highly significant between LD-1 and LD-3 (p=0.0012). Fig. 1.D. Total numbers of aphids in LD-1, 2 and 3. The aphid numbers were not significantly higher in any portion of the vine regions.

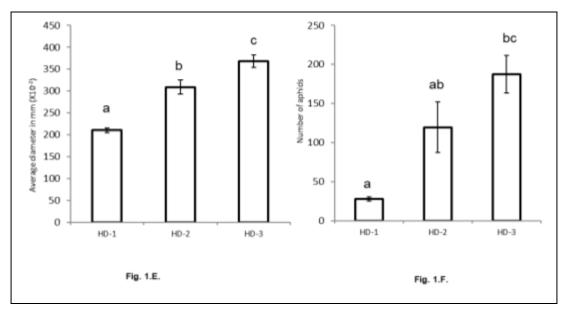


Fig 1: E.F. Aphid colony establishment within different segments in the higher diameter (HD) vines.

Fig. 1.E. All the vines in HD category were further considered for stem diameter recording. The 30 cm vines were segmented into three regions i.e., HD-1 (1^{st} 10 cm length from tip of the vine), HD-2 (2^{nd} 10 cm length from the end of HD-1), HD-3 (3^{rd} 10 cm length from the end of HD-2). In each HD-1,2 and 3 regions, the vine stem diameter was recorded in the midpoint. Three data from a single vine were recorded separately. The HD-1 vine diameter from all vines was considered for calculation. The vine diameter was found to be highly

significant between LD-1 and LD-3 (p=0.0008). Fig. 1.F. Total numbers of aphids in each HD-1,2 and 3 regions. The aphid numbers were significantly higher in HD-3 as compared to HD-1.]

Non-significant aphid population establishment within LD vines

The 30 cm vines from the tip were assigned 3 regions started from tip of the vine. There will be three segments within a

Differential aphid colony establishment within segments in the HD vines

The 30 cm vines from the tip were assigned three regions started from tip of the vine. There will be three segments within a single vine and abbreviated as HD-1, -2 and -3. Total insect population was counted in HD-1, -2 and -3. The diameter reading of each vine in HD-1, -2 and -3 was also considered. In the HD vines, there was significant difference (p=0.0214) of aphid number between HD-1 and HD-3 (Fig. 1.F). It was also found that there was significant difference in vine diameter between HD-1 and HD-3 (p= 0.0008) (Fig. 1.E). This data indicated that aphid proliferation requires for optimum diameter of vine for sucking the sieve element sap

and the diameter of HD-3 might satisfied the condition required to sufficient aphid sucking.

Differential aphid colony in the corresponding parts from LD and HD

Different segments (-1, -2 and -3) of both vines (LD and HD) were also showed the differential aphid colony measure. The HD-1 has significant aphid population as compared to LD-1 (P=0.0002) and positively correlated with the significant different in vine diameter (P=0.0001) (Fig. 2.A and B). In contrast, HD-2 does not support significant aphid population as compared to LD-2 (P=0.0789) although significant difference in vine diameter (P= 0.0005) (Fig. 2. C and D). The characteristic feature in this HD-2 vine was that there was high standard error in aphid population among the vines although p value was near significant (p value= 0.0789). In case of region number 3 in both vines (LD and HD), there was positive correlated with the vine diameter (P=0.0106) (Fig. 2. E and F).

[Fig. 2: Aphid colony establishment in term of total aphid number in LD-1 versus HD-1, LD-2 versus HD-2 and LD-3 versus HD-3.

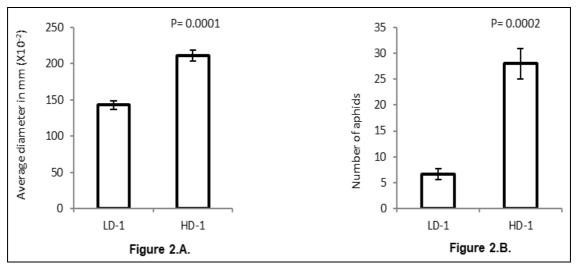


Fig 2: A.B. The diameter of HD-1 was significantly higher as compared to LD-1 (p=0.005). The higher diameter in HD-1 was positively correlated with total aphid number in HD-1 which was also significantly higher in numbers (p=0.0002).

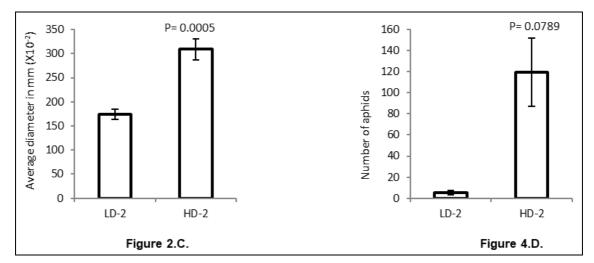


Fig 2: C.D. The diameter of HD-2 was significantly higher as compared to LD-2 (p=0.0005). The higher diameter in HD-2 was positively correlated with total aphid number in HD-2 which was not statistically significant (p=0.0789). The fluctuating aphid population within HD-2 was realized.

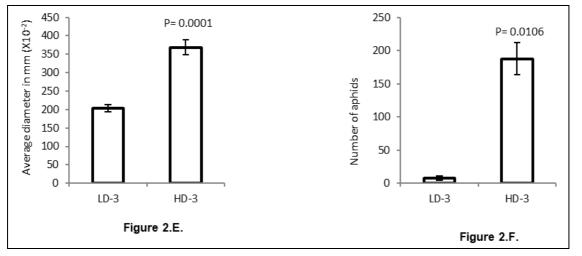


Fig 2: E.F. The diameter of HD-3 was significantly higher as compared to LD-3 (p= 0.0001). The higher diameter in HD-3 was positively correlated with total aphid number in HD-3 which was statistically significant (p=0.0106)]

Leaf petiole also supported differential aphid population Random collection from high diameter as well as low diameter leaf petiole gives differential aphid population establishment (Fig. 3.A.B). The selected leaf petioles have significant difference in diameter in HD leaf petiole as compared to LD leaf petiole (P=0.000). Characteristically, it was found that higher diameter of leaf petiole supports significant number of aphid population establishment (P=0.000). The aphid establishment in leaf petiole was measured in unit length of cm because the different length of petiole was observed and it was logical to measure aphid establishment in unit length.

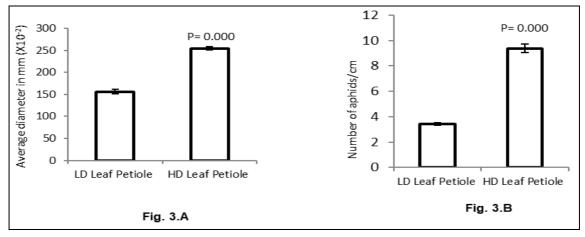


Fig 3: A.B Leaf petiole also supports differential aphid population establishment.

Fig. 3.A. The random collection of LD and HD leaf petioles showed the significant difference in petiole diameter. The significantly higher diameter leaf petiole in HD Leaf Petiole as compared to LD Leaf Petiole (p=0.000). Fig. 3.B. The total

aphid number was also higher in HD leaf petiole. The number of aphids in HD leaf petiole was significantly higher in HD leaf petiole (p=0.000).

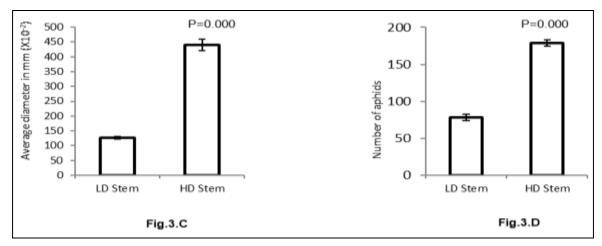


Fig 3: C.D. Higher stem diameter promotes more aphid colony establishment.

Fig. 3.C. The selected stem which was infested with aphid was significant in stem diameter difference between Lower and Higher Diameter (LD and HD) stem (p=0.000). Fig. 3.D.

The stem with significantly higher in diameter promotes higher number of aphid (p=0.000).

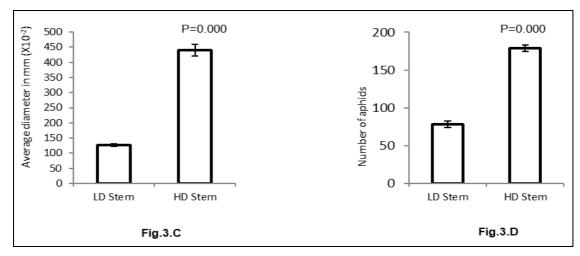


Fig 3: E.F. The distribution of aphid colony establishment within the leaf veins of *Dolichos lablab*. The total leaflet vein was divided into two equal segments. The leaflet diameter was taken at the base of the base of each segment.

Fig. 3.E. It shows the significant number of aphid's colony establishment in the 1^{st} part of the leaflet vein which was again in higher in diameter (p=0.000; Fig. 3.F) as compared to mid portion of the leaflet vein (Fig. 3.E. and p=0.000).

Higher stem diameter promoted more aphid colony establishment

The same trend was also observed in the stem from the aphid establishment point of view (Fig. 3.C.D). It was recorded that the higher diameter of vine stem promotes the aphid colony (P=0.000). It was also found that HD stem promotes more aphid proliferation as compared to LD stem (P=0.000). All the data support that aphid proliferation was positively correlated with diameter of vines, leaf petioles and stems.

More number of aphid establishment in the higher diameter region at the base of leaflet vein

In Fig. 3.E.F., it was significant establishment of aphid population in the 1^{st} part of the main leaflet vein. The same size leaflet was divided into two equal regions. In each region, the diameter was taken at the base of the leaflet vein. Fig. 3.E.F. showed that the characteristically a greater number of aphid's colony establishment (p=0.000) in the 1^{st} part of the leaflet vein which was again in higher in diameter (p=0.000) as compared to mid portion of the leaflet vein.

Discussion

Dolichos lablab belonged to the family *Fabaceae*. It was cultivated for vegetable purpose. It was also called hyacinth bean, dolichos bean, seim bean, lablab bean, Egyptian kidney bean, Indian bean, chicharo and Australian pea. In general, *Dolichos lablab* was an annual or short-lived perennial although the wild species was perennial. The stem length may be extended up to six meters. The leaves were made up of three pointed leaflets. It was a dual-purpose crop satisfying the pulse as well vegetable in India. Its tender and immature pods were also consumed as vegetable. The mature seed was also popular for consumption. This crop was also cultivated as fodder crop. It was recorded that as many as 55 species of insects and a species of mite attack this crop from seedling stage to the harvest stage (Govindan, 1974) ^[4]. The major challenge attributed for lower yields of *Dolichos lablab* has

been due to the damage caused by the insect pests.

Among the insect pests, the aphid, Aphis craccivora Koch was a serious issue in the cultivation of this crop. Aphis craccivora was the leguminous pest in general. Aphid only suck the nutrient rich phloem sap from tender shoots, inflorescence and pods resulting in induced sink that have great impact on down line effect like drying up of tender shoot and premature fall of flower buds, flowers and tender pods, stunted growth etc. Recent study indicated that aphid infestation re-programmed the physiology to produce more available nutrient molecules for fulfillment of the nutrient demand. In the present study, it was characteristically observed that variation in the vine diameter was present within the single plant. The aphid infested vines were selected randomly and group the vines on the basis of its diameter. The infested vines were classified into two groups like lower diameter (LD) which was less than 2 mm diameter and higher diameter (HD) which was higher than 2 mm diameter. Further observation was also indicated that aphid population establishment variation was correlated with the vine diameter. The average mean of LD diameter was calculated to be 1.712±0.072 whereas the mean diameter of HD vine was 2.96±0.15 (Fig. 1.A). The diameter difference was calculated to be significant (p=0.00; Fig. 1.A). Further study pointed out that the aphid counting showed total aphid number in 30 cm length vine was 20.17±3.97 in case of LD vine whereas aphid count was significantly higher in HD (avg.= 335±58; p= 0.007; Fig. 1.B). An aphid had two choices when landing in the plant. Aphid might move to different genotype due to 'antixenosis' effect (that causes the aphid's behavioral changes) coming from the present plant (Kogan and Ortman., 1978)^[9]. Antixenosis adversely impacted on insect behavior, for example its ability to find sieve elements, thus deterring infestation. The second option was that aphid might establish the stylet penetration for ingesting the phloem sap and continuing. The antibiosis, the plant resistance category in which aphid physiology was affected, resulted in adverse impact on the growth, development and/or, reproduction or survival of the insect (Smith, 2005)^[20]. After prolong sap sucking, aphid proliferation was observed due to higher volume of sieve element sap which was rich in simple sugars and amino acids (Mondal., 2017, 2020; Sengupta et al., 2010;

Chakraborti *et al.*, 2009; Louis *et al.*, 2012; Mondal *et al.*, 2012; Mondal., *et al.*, 2018) ^[18, 13, 21, 1, 6, 14]. This differential aphid population in HD vine as compared to LD vine was definitely due to better sap sucking and subsequent nutrition. Due to higher diameter of vine, somehow aphid enjoyed the HD-specific facilitation for establishing the success stylet penetration, salivation and better sucking, avoiding the plant defense mechanism etc.

The curiosity was extended to intra regions of both LD and HD vine to test its universality of diameter-based modulation of aphid population establishment. The LD-3 was significantly higher in diameter as compared to LD-1 but did not support the significantly higher number of aphids. The mean diameter of LD-3 region was 2.03 ± 0.079 mm whereas the diameter of LD-1 was 1.43 ± 0.051 mm (Fig. 1.C). The mean number of insect count was 8 ± 3.21 in LD-3 region where as it was 6.67 ± 1.02 in LD-1 (Fig. 1.D). So, the significant difference in diameter did not correlate with significant aphid establishment in LD vine. It could be proposed that higher diameter above the critical measure or facilitation thereof would be required for aphid proliferation in vine (Sharma *et al.*, 2017)^[19].

In case of HD vine, the both HD-2 and -3 was significantly higher in diameter as compared to HD-1 but does support the significantly higher number of aphids by HD-3 when compared to HD-1 region. The mean diameter of HD-3 region was 3.68 \pm 0.079 whereas the diameter of HD-1 was 2.11 \pm 0.054 (Fig. 1.E). The mean number of insect count was 187.5 ± 23.94 in HD-3 region where as it was 28 ± 2.94 in HD-1 (Fig. 1.E). So, the significant difference in diameter was correlated with significant aphid establishment in HD-3. There was an existence of significant difference in diameter between HD-1 (avg= 2.11 ± 0.054 mm; Fig. 1.E) and HD-2 (avg= 3.09± 0.16 mm; Fig. 1.E) but HD-2 did not support significant number of aphid proliferation as compared to HD-1. The mean number of aphid count in HD-2 was 119.5 \pm 32.41 (Fig. 1.F). From the data of both LD and HD vine solidified the concept that minimum diameter above the critical measure or 'facilitation thereof' would be promoting role for aphid proliferation in vine.

When the aphid as well as diameter of the same segments from both vines were compared, it was characteristically found that except segment -2, segment -1 and -3 shows the positive co-relation between aphid population establishment and vine diameter (Fig. 2.A-F). So, the higher diameter facilitated the aphid to suck more volume of sieve element sap either direct or indirect ways.

The same observation was also realized in leaf petiole where aphid infestation was observed in natural environment (Fig. 3.B). In case of LD petiole, the average number of aphids was 3.43 ± 0.1 per cm length whereas the average number of aphids in HD leaf petiole was 9.38 ± 0.34 per cm of length

(Fig. 3.B). This differentiated aphid establishment was correlated with differentiated measure of leaf petiole diameter (mean diameter of LD leaf petiole= 1.565 ± 0.0497 mm and mean diameter of HD leaf petiole= 2.55 ± 0.043 mm (Fig. 3.A). This trend was also applied to stem where significantly higher aphid population was found in HD stem as compared to LD stem (Fig. 3.C-D). The infested LD stem diameter was 1.27 ± 0.046 mm and the aphid infested HD stem diameter was 4.41 ± 0.019 mm which was significantly different (Fig. 3.C). The significantly different stem diameter impacted on aphid population establishment. The average number of aphid count was 78.33 ± 4.1 in LD stem whereas the aphid measure was quite high i.e., 178.67 ± 4.29 (Fig. 3.D). Even the diameter of the leaflet vein also modulated the aphid establishment along the vein length. The same leaflet vein diameter was significantly higher in diameter at the base position of the leaf as compared to the middle position of the leaflet vein. This characteristic feature differentiated the aphid establishment along the leaflet vein (Fig. 3.E-F). The aphid counted up to middle of the leaf was an average of 19.67 \pm 0.92 whereas rest part of the main leaflet vein supported an aphid average on 4.12 ± 1.3 (Fig. 3.F).

Based on observation, it was proposed a model presented in the Fig. 8. This model based on theme that more aphid colonization was dependent on more sieve element sap ingestion from phloem. Somehow, aphid could drink more sieve element sap in HD plant parts as compared to LD part. This could be due to 'HD-derived facilitation' that promoted aphid well feeding or suppression of plant defense response that make prolong sucking period in HD vine and that ultimately effected on more sap ingestion by the aphid as compared to LD plant parts. Aphid could face less difficulty in successful probing and salivation in HD plant part as compared to LD plant parts. Another reason may be that HD vines contained larger volume of sieve element as well as distance from the surface of the vine. The length of stylet may be optimally fitted to ingest the sieve element sap or due to larger volume of sieve element, aphid consumed more volume of sap within a fixed period of time as compared to LD plant parts. As a result, in the same period of interval, aphid proliferated more in the HD plant parts as compared to LD plant parts. The universal finding of a greater number of aphids in HD plant parts as compared to LD plant parts like inter vines, intra vine (only in HD vines), between same segment of HD and LD vines, stem, leaf petiole as well as leaflet veins (Fig. 3.A-F) might be definitely correlated with the vine or vein diameter that favored more aphid proliferation. This observation might be useful in resistance breeding in *Dolichos lablab* crop for developing resistant line because it carried a relevant significance in *Dolichos lablab* crop which was worse affected by Aphis craccivora infestation.

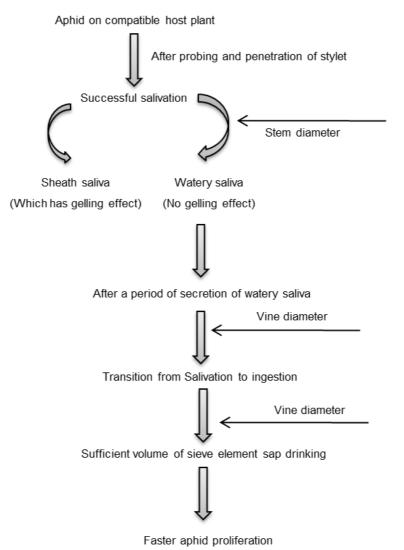


Fig 4: Generalized scheme of aphid colony proliferation and possible role of stem diameter

In smaller diameter vine, the total volume of sieve element sap ingestion could be lower as compared to higher diameter vine. The more sucking of sieve element sap contributed a higher aphid proliferation in the vine, stem, leaf petiole and leaflet vein. The higher diameter benefitted the successful stylet penetration, successful salivation, phase change from salivation to ingestion etc.

Conclusion

Aphids were dedicated sap-sucker and treated as agriculturally important pest in many agricultural crops. Aphid colonization drastically limited plant productivity due to the removal of nutrients that changes the source-sink relationships, the elicitation of plant responses that were deleterious to plant productivity and creation of induced sink at the site of aphid feeding (Goggin, 2007)^[3]. Due to their high reproductive rate, aphid increased their number within very short period of time that heightened their impact as agricultural pests. In addition to, many aphids were excellent vectors for diseases causing viruses, resulting in additional damage to the plants (Matthews, 1991)^[11]. In the resistance breeding, proper understanding of plant defense response to sucking insect was essential for developing resistant line in crop plant. In the present study, within the same plant the aphid colony distribution was not uniform and depends on diameter of vines, stem, leaf petiole and leaflet's vein. This

knowledge might be explored in resistance breeding in *Dolichos lablab* crop. To the best of my knowledge, this was the first report that showed the differential aphid colony development within the same plant was correlated to the diameter of various parts of plant.

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