IMPROVING SAMPLE PREPARATION AND DNA EXTRACTION METHOD IN APHIDS

ÎMBUNĂTĂȚIREA METODELOR DE PREGĂTIRE A PROBELOR ŞI DE EXTRACȚIE A ADN-ULUI LA AFIDE

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Abstract: *Improving the sample preparation, testing and improving DNA extraction methods in aphids were the aims of our paper. In this field, few researches were developed at national level, and relatively few titles are found in literature, too. Traditional sampling methods, in collection bottles were used. Sample preparation and DNA extraction were performed according to literature methodology, but technical improvements were also tested. The molecular approach of this field is relatively new and it has been developed in last decade. The improved methods obtained in aphid sample preparation and DNA extraction will facilitate the development of PCR based methods of genotyping the plant predators, globally, and development of screening methods for several interesting species, simultaneously. The importance of implementing these techniques at large scale is revealed by the accuracy of results, and also by the possibility of suitable selection of the products used for fighting against aphids.*

Rezumat: *Scopul lucrării de față îl reprezintă cercetările privitoare la îmbunătățirea metodelor de pregătire a probelor şi de extracție a AND-ului din populații de afide. În acest domeniu, au fost efectuate puține cercetări, iar literatura de specialitate este limitată. Pregătirea probelor şi extracția AND-ului s-au efectuat conform metodologiei recomandate de literatură. Abordarea moleculară aplicată în acest domeniu este relativ nouă şi progresele datează doar din ultimul deceniu. Îmbunătățirea metodelor de pregătire a probelor şi de extracție a AND-ului din afide va conduce la perfecționarea metodelor de genotipizare a dăunătorilor plantelor pe baza reacției PCR, în general, dar şi la dezvoltarea metodelor de evidențiere a mai multor specii, simultan. Importanța implementării acestor tehnici la scară largă este evidențiată de exactitatea rezultatelor, dar şi de perspectivele unei selecții mai eficiente a produselor de combatere a acestuit tip de dăunători ai plantelor.*

Key words: *aphids, DNA, sampling, extraction, improvement*
Cuvinte cheie**:** *afide, AND, sampling, extractie,îmbunătățire*

INTRODUCTION

Aphids are common pests of field crops fruit trees, vegetables, indoor and outdoor ornamental plants (e.g. cotton aphid - *Aphis gossypii*, crape myrtle aphid – green peach aphid, *Myzus persicae*, oleander aphid - *Aphis nerii*, podocarpus aphid - *Neophyllaphis podocarpi*, rose aphid - *Macrosiphum rosae* -, spiraea aphid - *Aphis spiraecola*, yellow rose aphid - *Acyrthosiphon (Rhodobium) porosum*, etc. Aphid populations are largest during the spring, on the flush of new growth. Reproduction is augmented by nitrogen levels and this element is particularly high in those portions of the plant that grow most rapidly. Aphids have piercing-sucking mouthparts and cause damage by sucking the plant juices. They are commonly found on the stems, undersides of leaves and on flower buds in colonies of individuals (DAY, E.R., 2002; OROIAN I., 2008). However, their ability to transmit plant virus diseases may be more harmful than any direct feeding damage. At the present time, there were identified over 1,375 species, worldwide.

In order to supply improved control (cultural, biological, chemical) the aphid genetics may be interesting (TSUCHIDA T. et al., 2002; FRANCIS F. et al., 2006). The correct field collection is another factor having crucial role in ensuring good quality, uncontaminated DNA. Research in the area of aphid DNA approached analyzes is poor. For this reason, we consider the aim of this paper - improvement of sample collection, testing and improving DNA extraction methods in aphids - of practice use.

MATERIAL AND METHODS

The aphid population was collected from the university green houses, where a large diversity of aphid population is recorded. The collection was performed individually, and a total number of 52 aphid individuals were harvested. The research was performed in the Laboratory of the Environmental Engineering and Protection Unit of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.

We tested several forms of capture systems, but finally we choose the group collection and we use typical sample bottles. Part of harvested samples were preserved in ethanol 80% and for others the freezing method was adopted for preservation (- 80^0C).

The use of the DNA classic extraction method was preferred against available kits, and cetyl trimethyl ammonium bromide (CTAB) extraction protocol (JUEN & TRAUGOTT, 2005) was implemented. The DNA purity and quantity were quantified using the UV molecular absorption spectrophotometry and NANODROP device (figure 1).

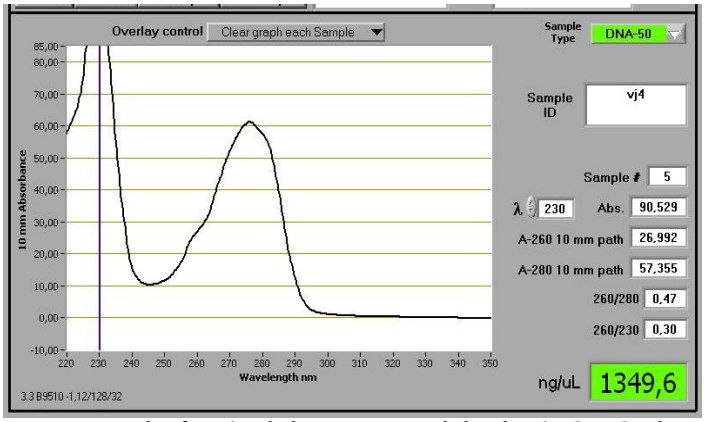

Figure 1. Example of DNA aphid spectrum recorded with NANODROP device

The statistical analyze was performed using STAT v. 6.0.

RESULTS AND DISCUSSIONS

The aphid DNA quantification was separately performed by storage method (table 1). Very small differences, statistically not significant ($p > 0.05$) were recorded between the average DNA quantities extracted from samples stored in two different conditions. Average DNA quantities of 792.21 ng/mL and 78.27 ng/mL were recorded in samples stored in ethanol 80% and samples frozen at – 80^0C, respectively. The same evolution was encountered in DNA purity, meaning an average of 1.79 in DNA stored in ethanol 80% and 1.75 in DNA stored frozen at – 80^0C.

These results show higher values for both quantity and purity of DNA preserved in ethanol 80% compared to frozen preservation.

Table 1

DNA quantity and purity in aphids stored in two different conditions (ethanol 80% and freezing at – 80^{0}C)

Storage method	Issue	n	Maximum	Minimum	$\overline{X} \pm s_x$
Ethanol 80%	DNA quantity (ng/µl)	26	1451.50	310.90	792.21 ± 27.81
	DNA purity	26	1.96	1.44	1.79 ± 0.02
Freezing at – 80^{0}C	DNA quantity (ng/µl)	26	1453.77	510.70	781.27 ± 25.32
	DNA purity	26	1.93	1.41	1.75 ± 0.03

An example of the distribution of aphid DNA individual quantity values (fig.1) reveal large variation from the expected values.

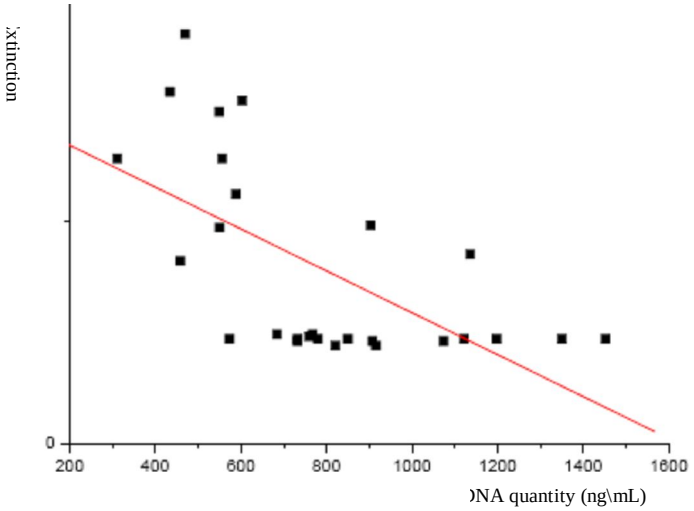

Figure 1. Distribution example of the aphid DNA individual values

Positive strong correlations (0.5776, and 0.5777, respectively) were reported between the aphid DNA quantities and extinctions in both storage conditions. They were statistically very significant, 792.21 ng/mL $e^{0.00002}$ ($p < 0.001$) in DNA stored in ethanol 80% and 781.27 ng/mL $e^{0.00001}$ ($p < 0.001$) frozen at – 80^{0}C (table 2).

Table 2

The correlation coefficients of DNA quantity in aphids stored in two different conditions (ethanol 80% and freezing at – 80^{0}C) and their significance

Storage method	Issue	n	$\overline{X}$	r	t	Significance
Ethanol 80%	DNA quantity (ng/µl)	26	792.21	0.5776	7.4884	***
Freezing at – 80^{0}C	DNA quantity (ng/µl)	26	781.27	0.5777	6.9951	***

The distribution of aphid DNA correlation values (figure 2) reveal small variation from the expected values.

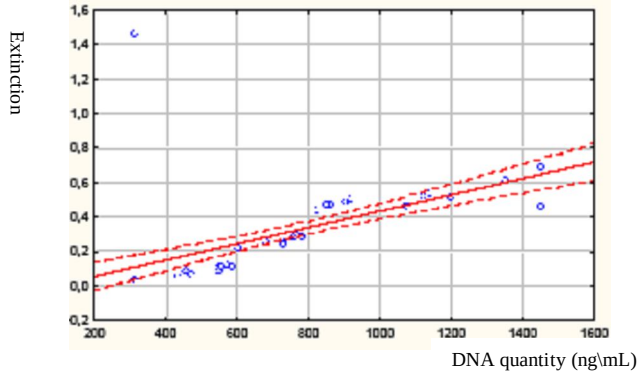

Figure 2. The correlation between extinction and average DNA purity from samples stored in two different storage conditions

CONCLUSIONS

1. Differences statistically not significant ($p > 0.05$) were recorded between the average DNA quantities extracted from samples stored in two different conditions (ethanol 80% and freezing at -80^0C).

2. The higher values reported for both quantity and purity of DNA preserved in ethanol 80% compared to frozen preservation, emphasize the advantage of using the ethanol preservation.

3. The distribution of aphid DNA individual quantity values reveal large variation from the expected values, but in spite of large variability the correlation distribution had small variation from the expected values. It reveals the low significance of sample variability in correlation relationships.

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