

MOLECULAR CHARACTERIZATION OF THREE VARIETIES OF *CONVOLVULUS ARVENSIS* AND THEIR TAXONOMIC RELATIONSHIPS

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Abstract

Perennial weed under study *Convolvulus arvensis* (field bindweed) highly distributed in all over the world. Because of its economic impact, *Convolvulus arvensis* is considered one of the ten 'world's worst weeds'. Preserve managers are most likely to find the weed on tracts once used for agriculture or in moist locations such as irrigated areas. Few studies have focused on the genetic DNA variation within and among varieties of species *Convolvulus arvensis*. Therefore, the aim of this study is the differentiation between three varieties of species *Convolvulus arvensis*, and determine the degree of relationships between them by molecular analysis.

In present study the RAPD is very helpful in confirm the difference between the three varieties belong to species *Convolvulus arvensis*, var *arvensis*, var *linearifolius* and var *nov.* (that need further studies). The classification is confirmed by study the finger print of the three varieties using RAPD-PCR technique, using 12 markers where 258 specific DNA -PCR bands as a result of ten of the used twelve primers.

INTRODUCTION

Convolvulus is derived from the Latin, *convolvere*, meaning to entwine, and *arvensis* means 'of fields' (Gray, 1970). Common Names: *Convolvulus* species are referred to as bindweeds. Both 'bindweed' and 'wild morning-glory' are applied to all the weedy species in the genus (Cox, 1915), but 'field bindweed' is used almost exclusively to refer to *Convolvulus arvensis* L. and is accepted by the Weed Science Society of America as the "official" common name (Whitesides, 1979). Other common names are 'possession vine,' 'creeping jenny,' 'creeping charlie' (Wiese and Phillips, 1976), 'field morning-glory,' 'orchard morning-glory' (Whitesides, 1979), 'European bindweed' (Swan, 1980), 'corn-bind,' 'morning-glory' (Callihan *et al.*, 1990), and 'small-flowered morning-glory' (Weaver and Riley, 1982). In Canada the name 'liseron des champs' (translation: field bindweed) may be used (Weaver and Riley, 1982). *C. arvensis* is a diploid Species $2n=2x=50$ (Walcott, 1937).

C. arvensis var. *obtusifolium* Choisy is generally the only variety recognized in North America (Robinson and Fernald, 1908). Gray (1970) refers to two forms of field bindweed: *C. arvensis* f. *cardifolius* Lasch., which has wide cordate leaves and broad basal lobes, and *C. arvensis* f. *auriculatus* Descr., which has linear, oblong or lanceolate blades with acute ear-shaped lobes at the leaf bases. Sixty or more varieties have been identified in Europe but the characteristics used to distinguish these have been attributed to environmental factors (Kogan, 1986). Plants with characteristics intermediate to those of these varieties are common in North America, further discouraging the use of the varietal names in the USA (Brown, 1946). *Convolvulus ambigens* is a hairy plant that has been grouped by Gleason (1952) with *C. arvensis*.

No previous record in Egypt for the varieties of *C. arvensis* (Täckolom, 1974 and Boulus, 2009).

A potentially more important use of RAPD techniques is the allocation of genotypes to specific heterotic groups which would reduce both cost and labour by eliminating intra-group crossing (Jain *et al.*, 1994). The development of the polymerase chain reaction (PCR) for amplifying DNA led to a revolution in the applicability of molecular methods. The most common version is RAPD analysis, in which the amplification products are separated on agarose gel in the presence of ethidium bromide and visualized under ultraviolet light (Williams *et al.*, 1990) Hashemi-Petroudi *et al.* (2010) state that the molecular markers technology provides novel tools for DNA fingerprinting of rice hybrids to assess hybrid seed purity. Kumar *et al.* (2010) used for the identification of *pigeonpea*, *Cajanus cajan* cultivars, the RAPD markers for the elucidation of genetic relationships.

MATERIALS AND METHODS

Plant material

Field collection was carried out from Helwan, Cairo, Giza and Khalubia governorates. The analysis established in Genetic Engineering Research Center, Agriculture. Research. Center, at (2006-2009).

DNA-electrophoresis (RAPD-PCR): RAPD-PCR was carried out according to the procedure given by Bagheri *et al.* (1995) with minor modification.

- a. **Isolation of plant genomic DNA:** Isolation of DNA from plants was carried out using CTAB method.
- b. **Preparation of DNA samples to precipitating DNA**
- c. **PCR Reactions:** Amplification reaction was carried out, the primer extension segment was extended to 5 min at 72°C in the final cycle.

Table 1. The amplification products resolved by electrophoresis.

Primer	Oligonucleotide
OPA -04	5-AATCGGGCTG-3
OPA -07	5-GAAACGGGTG-3
OPA -10	5-GTGATCGCAG-3
OPA -14	5-TCTGTGCTGG-3
OPB -01	5-GTTTCGCTCC-3
OPB -02	5-TGATCCCTGG-3
OPB -03	5-CATCCCCCTG-3
OPB -04	5-GGACTGGAGT-3
OPB -07	5- GGTGACGCAG-3
OPB -10	5-CTGCTGGGAC-3
OPB -15	5-GGAGGGTGT-3
OPC -07	5-GTCCCGACGA-3

d. Gel electrophoresis and visualization of DNA bands

Table (1): Decamer oligonucleotide primers used in PCR-RAPD techniques.

e- Photography of agarose gels

Agarose gels were examined on UV transilluminator filter by ultraviolet light (302 nm wavelength) to detect the ethidium bromide/DNA complex by using Polaroid film type 57 (ASA3000) (Sambrook *et al.*, 1989).

Data analysis:

RAPD bands were scored as 1(present) or 0 (absent) in a binary matrix for each primer. A conservation criterion for the selection of bands was used. Only reproducible and well fined bands were considered as potential polymorphic markers. Average taxonomical distance (DIST) was generated by using the similarity of interval data program (SIMINT) in order to measure the dissimilarity between the three varieties. Clustering was performed using the unweighted-pair-group method with arithmetic mean (UPGMA) by using sequential, agglomerative, hierarchial and nested clustering method (SAHN) as defined by Sneath and Sokal (1973). The output of SAHN-clustering program was present in the form of phenogram by using the tree display graphic (TREEG).

Statistical analysis:

An IBM compatible PC was used to store and analyze the data and to produce graphic presentation of important results. Calculations were done by means of statistical software package namely "SPSS".

RESULTS AND DISCUSSION

RAPD analysis technique has used extensively for varietal identification, phylogenetic relationships, parentage determination and marker assisted selection in a wide range of plant species because of its simplicity (Soliman *et al.* 2007). The sensitivity of the RAP-PCR technique was higher than the methods of fingerprinting in terms of the samples quantity and results quality (El-anany, 2002) Eppelen and Lubjuhn (1999), reported that DNA fingerprinting has been successfully applied to plants to develop genetic profiles. It has become an important tool in diverse fields of plant population research e.g. the study of breeding systems, genetic relatedness between or within species and populations, assessment of gene flow and gene identification.

RAPD analysis has been used in present investigation to differentiate between the three varieties under study. RAPD is acronym coined by William *et al.*, (1990) for Random Amplified Polymorphic DNA. The term random may somewhat misleading in that the only random component is the sequence of a primer rather than regions amplified.

More recently, PCR-based marker system have become available. One of these has been called RAPD-PCR (Newbury and Ford, 1993). In this method, short oligonucleotides of arbitrary sequence were used singly to support the amplification region of the test plant genome and amplification products are separated by gel electrophoresis. The amplified DNA fragments were refers to as RAPD markers. Differences between genotypes as reflected as differences in these banding patterns (Virk *et al.*,1995).

Primer OPA7 showed three major band at 1897, 801 and 220 bp for all studied varieties. Var. *arvensis* and *nov* have common bands at mol. wt. 3081 and 1614 bp. Var. *arvensis* showed five characteristic bands at mol. wt. 1614, 1529, 994, 892, 467 and 288 bp, while var. *nov* showed one band of 1049 bp.

Primer OPA10 showed ten major bands at mol. wts. 3822, 614, 1107, 942, 301, 719, 612, 580, 338 and 288 bp. One common bands for var. *linearifolius* and *nov*. at mol. wt. 443 bp in addition to one characteristic band at mol. wt. 2230 bp for var. *arvensis*, and one band at mol. wt. 1897 bp for var. *linearifolius* has, while var. *nov*. showed one band at mol. wt. 1233 bp.

Primer OPA14 showed four major bands at mol. wt. 1049, 801, 443, and 245 bp. Two common bands for both var. *arvensis* and *nov*. at mol. wt. 2621 and 1614 bp and one common for var. *arvensis* and var. *nov*. Var. *arvensis* showed four characteristic bands at mol. wts. 1449, 338, 288 and 220 bp. Var. *linearifolius* showed one band at mol. wt. 1301 bp, while var. *nov*. at mol. wt. 580 bp.

Primer OPB2 showed one major band at mol. wt. 528 bp. One common band for var. *arvensis* and var. *nov.* at mol. wt. 452 bp.

Primer OPB3 showed two major bands at mol. wts. 1336 and 849 bp. One common band for var. *arvensis* and var. *linearfolioides* at mol. wt. 476 bp. Var. *linearfolioides* showed one characteristic band at 931 bp while, seven bands for var. *nov.* at mol wts 2615, 2127, 1730, 1032, 797, 429 and 315 bp.

Primer OPB10 showed three major bands at mol. wts. 1407, 1032 and 884 bp. Two common bands for var. *arvensis* and var. *nov.* at mol. wts. 1643 and 616 bp. Var. *arvensis* have three characteristic bands at mol .wts 2359, 528, and 429 bp, while var. *nov.* showed two bands at mol. wts 1269 and 476 bp.

Primer OPA4, showed 10 major bands at mol. wts. 2342, 1994, 1444, 1229, 758, 580, 443, 304, 259 and 209 bp.

Primer OPB15 showed has six major bands at mol. wts. 2472, 1369, 891, 549, 598 and 304 bp. Var. *arvensis* showed four characteristic bands at mol wts 1046, 645, 467 and 339 bp while, two characteristic bands for var. *nov.* at mol. wts. 1791 and 1524 bp.

Primer OPB1 showed 10 major bands at mol. wts. 2726, 1935, 1433, 1315, 1157, 933, 857, 535, 432 and 258 bp.

Primer OPB7 showed six major bands at mol. wts. 1108, 821, 691, 535, 396 and 306 bp. Two common bands for var. *arvensis* and *linearfolioides* at mol. wts. 635 and 364 bp in addition to one at mol. wt. 1017 bp for var. *arvensis*, one for var. *arvensis* and var. *nov.* at mol. wt., 933 bp, one band for var. *linearfolioides* and var. *nov.* at mol. wt. 1062bp.

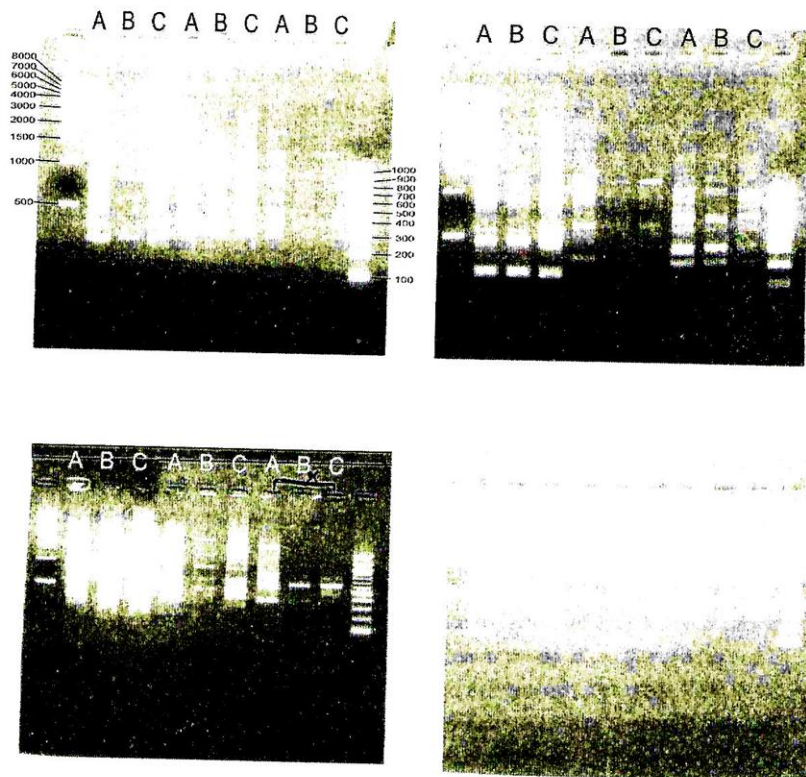
According to the present results of RAPD-PCR for var. *nov* which showed some specific bands of 1049 bp in case of using the OPA7 primer, 1233 bp in case of using OPA10 primer, 580 bp in case of using primer OPA14, seven bands at mol. wts. 2615, 2127, 1730, 1032, 797, 429 and 315 bp in case of using primer OPB3, two bands at mol. wts. 1269 and 476 bp in case of using primer OPB10 and two characteristic bands at mol. wts. 1791 and 1524 bp in case of using primer OPB15, rather than the other two varieties which indicate that *Convolvulus* var. *nov* may consider as one new variety. In this respect, additional results for anatomical and morphological using Scanning Electron Microscope features may be evaluated.

The resulting dendrogram (fig.2), show that var. *linearfolioides* is separated from the other varieties at 0.76 bp. The var. *arvensis* and var. *nov.* separated at 0.78bp.

According to the present results of RAPD-PCR for var. *nov* which showed some specific bands of 1049 bp in case of using the OPA7 primer, 1233 bp in case of using OPA10 primer, 580 bp in case of using primer OPA14, seven bands at mol. wts. 2615,

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2127, 1730, 1032, 797, 429 and 315 bp in case of using primer OPB3, two bands at mol. wts. 1269 and 476 bp in case of using primer OPB10 and two characteristic bands at mol. wts. 1791 and 1524 bp in case of using primer OPB15, rather than the other two varieties which indicate that *Convolvulus* var. *nov* may consider as one new variety. In this respect, additional results for anatomical and morphological using Scanning Electron Microscope features may be evaluated.



Fig(1): RAPD PCR pattern of three different varieties of *C. arvensis*(A=var. *arvensis*, B=var. *linerfolius*, C= var. *nov*)

Table 2. RAPD PCR analysis of three different varieties of *C. arvensis* (A=var. *arvensis*, B=var. *linerfolius*, C= var. *nov*)

	Total MW	Var. <i>arvensis</i>	Var. <i>linerfolius</i>	Var. <i>nov</i>
OPA 4	2342	1	1	1
	1994	1	1	1
	1444	1	1	1
	1229	1	1	1
	758	1	1	1
	580	1	1	1
	443	1	1	1
	304	1	1	1
	259	1	1	1
	209	1	1	1
	2621	1	0	1
	1614	1	0	1
OPA 14	1449	1	0	0
	1301	0	1	0
	1049	1	1	1
	801	1	1	1
	612	1	1	0
	580	0	0	1
	443	1	1	1
	338	1	0	0
	288	1	0	0
	245	1	1	1
	220	1	0	0
	3081	1	0	1
OPA 7	2230	1	0	1
	1897	1	1	1
	1614	1	0	1
	1529	1	0	0
	1301	1	0	1
	1049	0	0	1
	994	1	0	0
	892	1	0	0
	801	1	1	1
	521	0	1	1
	467	1	0	0
	288	1	0	0
OPA 10	220	1	1	1
	3822	1	1	1
	2230	1	0	0
	2002	0	1	0
	1897	0	0	1
	1614	1	1	1
	1233	0	0	1
	1107	1	1	1
	942	1	1	1
	801	1	1	1
	719	1	1	1
	612	1	1	1
580	1	1	1	
443	0	1	1	
338	1	1	1	
288	1	1	1	
528	1	1	1	
452	1	0	1	
OPB3	2615	0	0	1
	2359	1	1	0
	2127	0	0	1
	1730	0	0	1
	1336	1	1	1
	1032	0	0	1
	931	0	1	0
	797	0	0	1
	649	1	1	1
	476	1	1	0
	429	0	0	1
	1643	0	0	1
OPB 10	315	1	0	0
	2359	1	0	1
	1407	1	1	1
	1269	0	0	1
	1032	1	1	1
	884	1	1	1
	616	1	0	1
	528	1	0	0
	476	1	1	1
	429	0	0	1
	387	0	1	1
	2472	1	1	1
OPB15	1791	0	0	1
	1524	0	0	1
	1369	1	1	1
	1046	1	0	0
	891	1	1	1
	645	1	0	0
	549	0	1	1
	467	1	0	0
	398	1	1	1
	339	1	0	0
	304	1	1	1
	2726	1	1	1
OPB 1	1935	1	1	1
	1433	1	1	1
	1315	1	1	1
	1157	1	1	1
	933	1	1	1
	857	1	1	1
	535	1	1	1
	432	1	1	1
	258	1	1	1
	1108	1	1	1
	1062	0	1	1
	1017	1	0	0
OPB 7	933	1	0	1
	821	1	1	1
	691	1	1	1
	635	1	1	0
	535	1	1	1
	396	1	1	1
	364	1	1	0
	306	1	1	1

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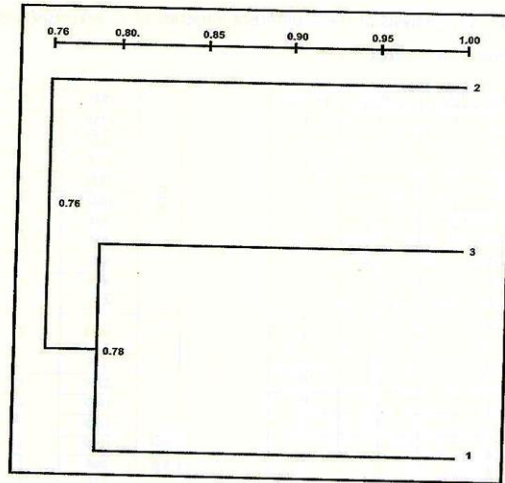


Fig (2) show phylogenetic tree Using UPGAMA method :

REFERENCES

1. Bagheri, A., J.G. Paul, P. Ianngridge and S. Rathjan. 1977. Genetic distance detected with RAPD- markers among selected *Australian* commercial varieties / and boron- tolerant exotic germplasm of pea (*Pisum sativum* L.). *Molecular Breeding*, 1: 193-197.
2. Bolous, L. 2009. Flora of Egypt. Cairo. Egypt. Al Hadara Publishing. 249.
3. Brown, E.O. 1946. Notes on some variations in field bindweed (*Convolvulus arvensis* L.). *Iowa State College J. of Sci.*, 20:269-276.
4. Callihan, R.H., C.V. Eberlein, J.P. Mc Caffrey and D.C. Thill. 1990. Field bindweed: Biology and management. University of Idaho, Cooperative Extension System, College of Agriculture Bulletin, 719.
5. Cox, H.R. 1915. The eradication of Bindweed, or Wild Morning-glory. U.S. Department of Agriculture Farmer's Bulletin, 368. Government Printing Office, Washington, D.C.
6. El-anany, M. F. 2002. Studies on Genus *Cuscuta* L. in Egypt, M.Sc. Thesis, Faculty of Science, Helwan University, Cairo, Egypt.
7. El-Nady, G.H. 2000. Molecular Genetic identification of endogenous. Barley cultivars. M.Sc. Thesis, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.
8. Gleason, H.A. 1952. The New Britton and Brown. Illustrated Flora of the Northeastern United States and Adjacent Canada. New York Botanical Garden, Bronx, NY.
9. Gray, A. 1970. Gray's Manual of Botany, a handbook of the flowering plants and ferns of central and northeastern United States and adjacent Canada, 8th ed. D. VanNostrand Co., New York.
10. Hashemi-Petroudi, S. H., S. A. M. M. Maibody, G. A. Nematzadeh and A. Arzani. 2010. Semi-random PCR markers for DNA fingerprinting of rice hybrids and their corresponding parents. *Afr. Jour. Biotechnology*, 9: 7, 20, 979-985.
11. Jain, A., S. Bhatia, S.S. Banga, S. Prakash and M. Lakshmikumar. 1994. Potential use of random amplified polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard (*Brassica juncea*) and its relationship to heterosis. *Theo. and App. Gen.*, 88: 116-122.
12. Kogan, M. 1986. Eco-physiology and control of *Convolvulus arvensis* L. in Ecology and Control of Perennial Weeds in Latin America. Food and Agriculture Organization of the United Nations, Rome.

13. Kumar, D. R., Y. A. Deshmukh and S. Tejbhan. 2010. Phylogenetic relationships of pigeonpea (*Cajanus cajan*) and its wild relatives based on RAPD markers, *Asian Jour. Bio. Sci.* 4 (2): 289-29
14. Pauls, K. P. 1996. Molecular markers: fast track screening for crop diversity, desirable traits. *Agri. Res. In Ontario*, 19(2): 7-9.
15. Robinson, B.L. and M.L. Fernald. 1908. Gray's New Manual of Botany. 7th ed. American Book Co., New York.
16. Sa'ad, F. 1967. *The Convolvulus species* of the Canary Isles, the Mediterranean region and the Near Middle East. Bronder-Offset, Rotterdam, 13-33.
17. Sneath, P.H.A and R.R. Sokal. 1973. Numerical Taxonomy. Freeman, San Francisco, California.
18. Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. Gel electrophoresis of DNA in Molecular cloning: A laboratory manual Cold Spring Harbor relationship to heterosis. *Theo. and App. Gen.*, 88: 116-122.
19. Soliman, M.S.A., A.A. Shehata, S.M. El-Dareir, F.A. Ghareeb and A.E. Shabasy. 2007. Molecular characterization of some Egyptian olive (*Olea europaea* L.) cultivars and their taxonomic relationship. *Egypt.J. Gene. Cytol.* 36:227-237.
20. Swan, D.G. 1980. Field bindweed, *Convolvulus arvensis* L. Washington State University, College of Agriculture Research Center, Bulletin pp 888.
21. Tandon, S. L. and C.P. Mzalik. 1959. Intraspecific Polyploidy and Evolution of diverse morphological forms in *Convolvulus pluricaulis* Choisy. *Nature*, 1: 184-481.
22. Täckholm, V. 1974. Student's flora of Egypt. *Cairo Univ.* 430.
23. Weaver, S.A. and W.R. Riley. 1982. The biology of Canadian weeds. 53. *Convolvulus arvensis* L. Canadian Journal of Plant Science, 62: 461-472.
24. Walcott, G.B. 1937. Chromosome numbers in the Convolvulaceae. *Amer. Nat.* 71: 190-192.
25. Whitesides, R.E. 1979. Field bindweed: A growth stage indexing system and its relation to control with glyphosate. PhD. thesis. Oregon State University, pp 76.
26. Wiese, A.F. and W.M. Phillips. 1976. Field bindweed. *Weeds Today*. 7:22-23.
27. Williams, J.G.K, A.R. Kublik, K. J. Livak, J.A. Rafalski and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, 18: 6531-6535.

التوصيف الجزيئي لثلاث أصناف من نوع العليق *Convolvulus arvensis* و
علاقتها بالصفات التقسيمية

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فى دراسة بمعمل بحوث الحشائش ، معهد بحوث المحاصيل الحقلية، ومعمل بحوث الهندسة الوراثية مركز البحوث الزراعية بالجيزة، مصر، خلال الفترة من ٢٠٠٥ وحتى ٢٠١٠م، تم تأكيد الدراسة التصنيفية لثلاث أصناف من نبات العليق باستخدام البصمة الوراثية بطريق تفاعل التسلسل والبلمرة (RAPD-PCR) وباستخدام ١٢ بادئ فى الحمض النووى DNA. أظهرت الدراسة وجود تباين بين الأصناف الثلاث من حيث عدد الشرائط الناتجة وأوزانها الجزيئية. أظهرت الدراسة الفروق الوراثية لأصناف العليق أرفينسيس من صنف أرفينسيس، لينيافولويس ونوف، وقد تأكدت النتائج باستخدام البصمة الوراثية من خلال ٢٥٨ شريط جزيئى لحمض DNA نتيجة استخدام عشرة بوادئ بتفاعلات البلمرة المتسلسل.