RETROTRANSPOSON DIVERSITY IN ARTICHOKE AND CARDOON (CYNARA CARDUNCULUS L.) AS REVEALED BY S-SAP PROFILING

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Summary
We describe the cloning of LTR retrotransposon-like sequences, and their use for Sequence-Specific Amplified Polymorphism (S-SAP) profiling in *Cynara cardunculus* L. Our results show that the developed assay is an useful tool for revealing genetic diversity within the species.

Key words
Globe artichoke, cardoon, S-SAP, retrotransposons.

Introduction

Cynara cardunculus L. belongs to the *Asteraceae* family (*Compositae*) and includes three taxa: globe artichoke (*C. cardunculus* L. var. *scolymus* L.), cultivated cardoon [*C. cardunculus*. L. var. *altilis* (DC)] and wild cardoon [*C. cardunculus* L. var. *sylvestris* (Lamk) Fiori]. Retrotransposon-based markers represents a useful tool for germplasm diversity studies. The mobility of retrotransposons within a genome allows them to be targeted for a PCR-based marker system referred to as S-SAP (Sequence-Specific Amplified Polymorphism, [1]), which generates fingerprinting profiles informative for genetic diversity and linkage analyses. Here we report: (i) the isolation from *C. cardunculus* of sequences related to Ty1-*copia*; (ii) the development of a modified S-SAP, based on LTR sequence; (iii) the application of S-SAP for the analysis of polymorphisms in *C. cardunculus*.

Material and Methods

Twelve *C. cardunculus* accessions were used for S-SAP profiling; these consisted of eight globe artichoke entries, representing the range of genetic variation in cultivated germplasm [2], two cultivated cardoons and two wild cardoons, sampled from natural stands in Sicily and in Sardinia.Thirty clones from a partial genomic library [3], were analysed using CENSOR software (www.girinst.org/Censor_Server-Data_Entry_Forms.html). One clone, *CYRE*-1, showed homology to a retro-element sequence (Figure 1). Longer retro-element sequences were identified following a modification of the protocol published elsewhere [4], using the artichoke RNAseH primers.S-SAP profiles were generated by digesting genomic DNA with, alternatively, *Eco*RI or *Taq*I enzymes and ligating universal adapters. S-SAP amplicons were generated using *CYRE*-39for IRD^{700} labelled primer and an adapter directed primer; PCR products were generated using the protocol reported in [5] and separated on a DNA analyser Gene ReadIR 4200 (LI-COR).
Genetic similarity among accessions was calculated according to Jaccard's Similarity Index and used to construct a dendrogram based on UPGMA.

Results and Discussion

CENSOR analysis identified one genomic artichoke sequence with partial homology to *copia*-like retrotransposons. Its 497bp sequence contained the terminal part of the RNAseH gene (96 putative amino acids), including the two conserved oligopeptide domains KTKHID and ADLFTK, a stop codon, a polypurine tract (PPT) and 48bp of a putative 3' LTR sequence (Figure 1). Phylogenetic analysis revealed its sequence to have a high level of similarity to *SZ*-55 and *OPIE*-2, from rice and maize respectively. The element was named *CYRE*-1 (*CYnara* REtro-element 1). The LTR walking procedure led to the isolation of three novel sequences (*CYRE*-39, *CYRE*-41 and *CYRE*-15) containing putative LTRs; *CYRE*-39 LTR was used to generate S-SAP profiles.

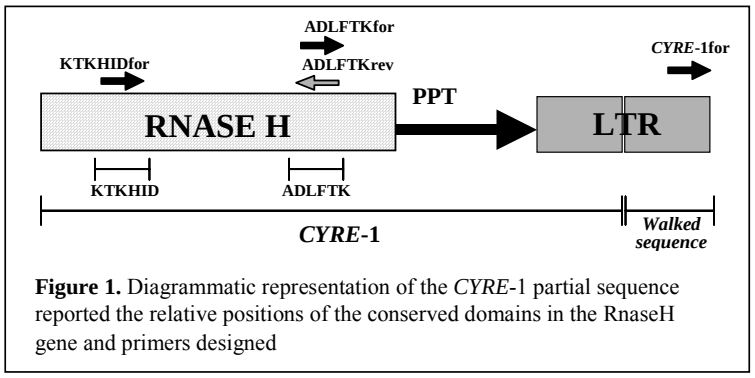

Figure 1. Diagrammatic representation of the *CYRE*-1 partial sequence reported the relative positions of the conserved domains in the RnaseH gene and primers designed

The two restriction enzymes used for S-SAP (*Eco*RI and *Taq*I) generated, respectively, 51 (57%) and 30 (39%) polymorphic bands when tested on the eight globe artichoke accessions; the average number of polymorphic bands per primer combination was 12.8 (range 8-18) for *Eco*RI and 7.5 (range 5-10) for *Taq*I. The number of polymorphisms increased to 100 (72%) and 57 (54%) when wild and cultivated cardoon accessions were included. The wild cardoon accessions clustered together, emphasising its significant differentiation from both cultivated forms (Figure 2), as previously detected using both AFLP [2] and SSR markers [6]. Globe artichoke and cultivated cardoon also formed separate clusters, but at a lower level of genetic differentiation as previously reported [2,6]. The S-SAP assay developed showed to be effective for the identification of retrotransposon-based DNA polymorphisms in *C. cardunculus*, and seems to be a promising tool for germplasm characterisation and conservation programs.

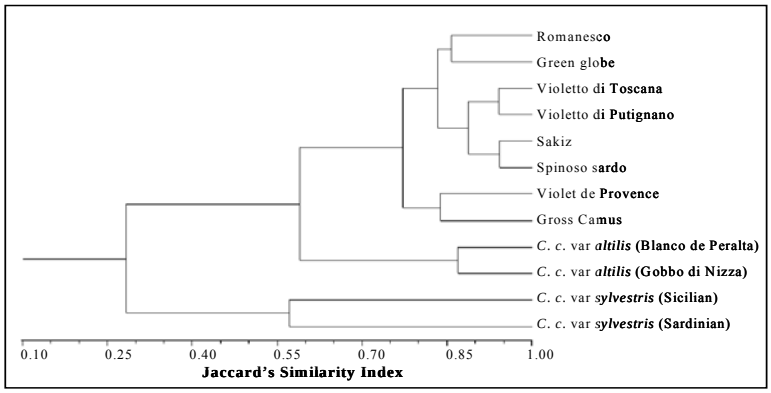

Figure 2 Dendrogram obtained from UPGMA cluster analysis of S-SAP data generated by the *Taq*I primers in combinations with *CYRE*-39for specific primer.

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RELATIONSHIPS AND GENETIC DIVERSITY OF GRAPEVINE (VITIS VINIFERA L.) GROWN IN ALGERIA AND IN MEDITERRANEAN BASIN

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Summary
Algeria represents a great resource of almost unknown genetic diversity for all the Mediterranean species, in particular for grapevine (*Vitis vinifera* L). Often, different local names were given to plant material that resulted identical from the genetic point of view. In this work 12 SSR markers were used to study the relationships and the genetic diversity among 60 autochtonous and cultivated grape varieties coming from the Mediterranean Basin.

Keywords
genetic characterization, DNA, microsatellite markers, SSR, synonymy

Introduction

Algeria has a long viticulture history and a rich tradition due to the settlement of many populations and civilizations. For many centuries, this situation made of Algeria the place of choice for the production and exchange of plant material [1]. This inheritance has led to the existence of a quite large grapevine germplasm, but also to the occurrence of cases of homonymy and synonymy. At present, it is widely acknowledged that DNA markers represent a significant resource for creating genetic and physical maps, distinguishing individuals and investigating genetic relatedness. Since the first application of SSR (Simple Sequence Repeat) markers in the grapevine identification, microsatellites have increasingly become quite the ideal tool for genetic studies due to their high polymorphism and the ease with which they can be detected. In this context, for the first time SSR markers were used to study the relationships and genetic diversity among wild and cultivated grapevine cultivars coming from Algeria and the Mediterranean Basin.

Material and Methods

Grapevine accessions from the two Algerian collections of Benchicao (Medea) and Mascara, and from the French collection of Montpellier (INRA) were analysed. Total genomic DNA was extracted from young leaves using the procedure described by [2] and sixty DNA samples were analysed at 12 SSR loci (Table 1) characterized by [3], [4] and [5]. The PCR was performed on 20 µl of a mixture containing: 50 ng DNA, 0.5 U AmpliTaq Gold polymerase (Applied Biosystems, USA), 2 µL GeneAmp 10x PCR buffer (Applied Biosystems), 1.6 mM $MgCl_2$, 200 µM dNTP and 0.5 µM of each primer, using a MJ Research PTC 100 thermal cycler. Amplification cycles consisted of an initial step at 95°C for 9 min, followed by 26 cycles of denaturation (50 sec at 95°C), annealing (45 sec at 50°C) and extension (90 sec at 72°C); a final elongation step was done at 72°C for 45 min. PCR-amplicons were analysed on a sequencing gel using an ABI-PRISM 377 DNA sequencer (Applied Biosystems). Data were analysed with the GENESCAN software (Applied

Biosystems) and alleles were defined by their size, determined in base pairs by comparison with the size standard. Statistical analyses were performed using the software POWER MARKER v 3.22 (http://www.powermarker.net).

Results and Discussion

The microsatellite loci chosen for this study, discriminated 34 different genotypes in 60 analysed cultivars. The genetic variability of this germplasm (Table 1) was evaluated on the basis of the number of alleles (mean: 9.1), gene diversity (GD: 0.79), observed heterozigosity (Ho: 0.80), and polymorphism information content (PIC: 0.77). These data indicated the presence of a high genetic variability in the local germplasm, comparable to the variability found in the Spanish grape germplasm [6]. Total probability of identity for the 12 loci was 6.67×10^{-15}, thus, cultivars with identical genotypes were considered synonyms. Among the investigated cultivars, DNA profiles showed the following synonymies: Ahmeur bou ahmeur, H. de Mascara, and Bordji; Chaouch rose and Chaouch; Amokrane and Amelal; Regina dei Vigneti and Dattier de Beyrout.

Locus	N° of genotype	N° of alleles	GD	Ho	PIC
VvS2	20	11	0.864	0.967	0.849
VvS5	18	11	0.828	0.655	0.807
VvMD5	24	8	0.837	0.900	0.816
VvMD7	16	9	0.774	0.883	0.745
VvMD24	13	7	0.659	0.483	0.620
VvMD27	15	7	0.764	0.767	0.735
VvMD31	18	8	0.821	0.800	0.797
VvMD36	17	12	0.814	0.767	0.792
VrZAG21	16	7	0.785	0.800	0.755
VrZAG62	19	9	0.783	0.850	0.755
VrZAG67	20	10	0.798	0.967	0.775
VrZAG 79	22	10	0.795	0.783	0.770
Mean	18.2	9.1	0.794	0.802	0.768

Table 1 - Number of genotypes, number of alleles, gene diversity (GD), observed heterozygosity (Ho), polymorphism information content (PIC) of 12 SSR loci studied in 60 *V. vinifera* cultivars.

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ISOLATION AND CHARACTERIZATION OF NUCLEAR MICROSATELLITE MARKERS IN DATE PALM (PHOENIX DACTYLIFERA L.)

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Summary
Simple Sequence Repeats were isolated from a genomic library of *Phoenix dactylifera* L. All primer pairs produced an amplification product of the expected size and detected high polymorphism among the analysed samples. SSR genetic markers are expected to be a very effective tool for evaluating genetic diversity in date palm germplasm. Cross-transferability in different species and genera was evaluated.

Keywords
Molecular markers, fingerprinting, polymorphism, SSR, DNA

Introduction

Date palm (*Phoenix dactylifera* L.) is the major fruit crop of arid climate region, cultivated mainly in North Africa but also in South Asia, in USA and in Australia. It covers a surface of about 800.000 ha and it is important – directly or indirectly – for the life of about 100 millions of inhabitants.
Random amplified polymorphic DNA (RAPD) markers have been used for the characterization of *Phoenix dactylifera* L. cultivars [1], even if they require an accurate standardization of the analysis to give reproducible patterns and they can be difficult to interpret due to their dominant mode of inheritance. In comparison, simple sequence repeats (SSR) have the potential to provide a more reliable method for DNA fingerprinting because of their high degree of polymorphism, co-dominant inheritance and the high reproducibility of the analysis.
The present research reports the isolation and the characterization of microsatellite sequences in *Phoenix dactylifera* L. and the study of the cross-transferability of these markers in other palm species.

Materials and Methods

Total genomic DNA was extracted from frozen leaves using a modified method of Thomas *et al*. [2]. Date palm genomic DNA was digested with RsaI and then used to construct a (GA)n and a (GT)n microsatellite-enriched library. SSR-containing DNA fragments were sequenced on semi-automatic sequencers. A set of 14 polymerase chain reaction (PCR) primer pairs for microsatellite amplification was designed using the software Primer Express (Applied Biosystems - USA). The PCR amplification was performed on 28 DNA samples of *P. dactylifera* from two Algerian Oasis (Biskra and Golea) and the USDA-ARS National Germplasm Repository (California). Cross-species amplification was tested on individuals of 9 other *Phoenix* species as well as of 10 other palm genera. The PCR products of each individual were analysed following Akkak *et al.* [3]. The PCR amplification was performed

on 50ng of DNA in a 20 µl final volume of a mixture containing 0.5 U TaqDNA polymerase (AmpliTaq Gold, Applied Biosystems), with the supplied reaction buffer, 1.5 mM MgCl2, 0.5 µM of each primer and 200 µM of each dNTP. The PCR programme was an initial denaturation at 95°C for 9 min, 28 cycles of 95°C for 30 sec, 50-58 °C for 30 sec, 72 °C for 1 min and a final elongation at 72°C for 45 min.

Results and Conclusion

All DNA samples were amplified at the 14 loci and analysed on ABI PRISM 377. They showed at least 1 or 2 alleles per locus.
Across the genus *Phoenix*, all loci revealed clear SSR patterns and polymorphism within the expected allelic range in most species.

We evaluated in details 14 loci and 8 of them were particularly interesting (Table 1) considering their good polymorphism.

The isolation and the characterization of nuclear microsatellite markers in date palm and the study of their cross-transferability is still in progress.
The criteria followed to select the primers will allow to carry out multiplex analysis and, therefore, to reduce time and cost of the technique.

Tab. 1 - Parameters of 8 loci used to study 28 date palm cultivars

Locus	Repeat type	Label	Allele size	Opt. T.a. (°C)
PDCAT1	$(TC)_{21}$	6-FAM	100	54
PDCAT 5	$(AG)_{16}$	6-FAM	158	54
PDCAT6	$(CA)_{14}(GA)_{23}$	HEX	180	54
PDCAT8	$(TC)_{16}$	NED	230	54
PDCAT 10	$(TC)_{16}$	6-FAM	111	54
PDCAT 11	$(TC)_7(TC)_{20}$	6-FAM	146	54
PDCAT 13	$(TC)_{21}$	NED	101	54
PDCAT 14	$(TC)_{19}(TC)_{16}$	6-FAM	131	54

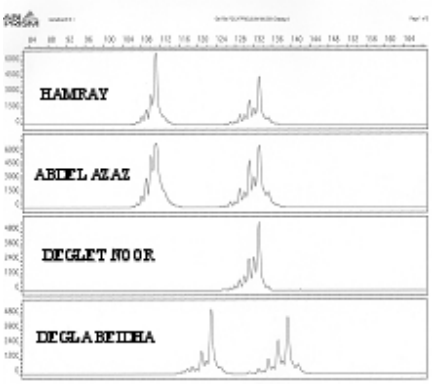

Fig. 1 – Polymorphism of PdCAT 1 locus and determination of a possible case of synonymy ('Hamray' and 'Abdel azaz')

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APPLICATION OF ANIMAL BIOTECHNOLOGIES TO SUSTAINABLE DEVELOPMENT OF LIVESTOCK FARMING IN WEST AFRICA

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Summary
Animal genetic resource knowledge is the first step of any genetic improvement programme. Studies, made by the *Centre International de Recherche-Développement sur l'Elevage en zone Subhumide* (CIRDES), have been consisted in an inventory analysis of West African cattle breeds, in their molecular characterization and in the analysis of the karyotype in order to assist any genetic conservation strategy plan. Serial Analysis of gene Expression (SAGE) technology is being used and genes that could be involved in trypanotolerance will be describing soon.

Keywords
Biotechnology, Biodiversity, Cattle breeds, Chromosome, CIRDES, Microsatellite

Abstract
In the framework of CIRDES research-development activities, numerous biotechnologies (PCR and related techniques, cytogenetics etc.) are used. The global objective is to improve cattle breeding and health. Thus, some microsatellite alleles have been found to be significantly associated with local cattle breeds; they highly contribute to the better understanding of the philogenetic relationships between cattle breeds (figure 1).

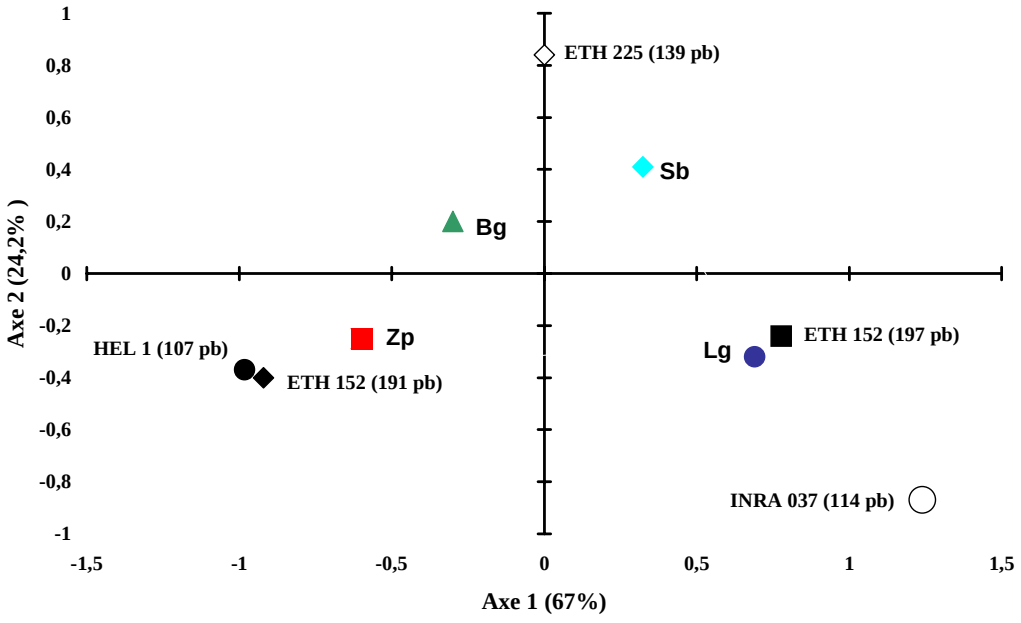

Figure 1: Relationship between microsatelite alleles and cattle breeds
Bg = Borgou; Lg = Lagune; Sb = Somba; Zp = Zebu peul;

Chromosome anomalies (1/29-roberstonian translocation), that decrease fertility, were discovered amoung several cattle populations (table 1) and adequate measures had been proposed to the breeders. This participates in the genetic improvement of local cattle and to the conservation of animal genetic resources.

Table 1 Prevalence of 1/29-robertsonian translocation

Cattle breed	Experimental station condition		Indigenous condition	
	Sampling size	1/29-robersonian translocation	Sampling size	1/29-robersonian translocation
Lagoon	80	0.0 (0%)	-	-
Somba	-	-	125	13 (10.4%)
Borgou	119	20 (16.8%)	33	Bad mitosis
Zebu	18	0 (0%)	75	2 (2.7%)

In the reproduction biotechnologies field, CIRDES is working on the control of the cattle sexual cycle. The artificial insemination techniques, the techniques of multiple ovulations and embryos transfer are used to assist periurban cattle breeding (milk and meat purposes). A bank with more than 10,000 doses of semen of a few local cattle breeds is available. This participates in the genetic improvement of local cattle breeds and in the genetic resources preservation.

So, biotechnology research will be useful in the biodiversity conservation and in the improvement of animal production and health. Urgently, the available new technologies must be transferred to Developing countries research centers. Indeed, the domain of application of biotechnologies to livestock breeding is wide and has enormous potentials. When new technologies are rationally used to complement traditional approaches, this will obviously result in sustainable increases in livestock productivities.

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PRESERVATION AND MANAGEMENT OF BIODIVERSITY IN FOREST SEED PRODUCTION CHAIN

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Summary
Genetic variability is of utmost importance for forest tree species, being linked with adaptability. However, ordinary procedures adopted in seed production chain often give rise to consistent loss of biodiversity from the seed source to the material used for reforestation. The reduction in genetic variability was studied in a Scots pine seed production chain and suggestion for a more efficient germplasm management are given.

Keywords
Genetic variability, Forest seed production, Molecular markers, *Pinus sylvestris*,Reforestation

Contribution
It is well-known the importance of genetic variability for forest species and the correlation between the latter and adaptability [1, 2]. As a consequence, the higher the genetic variability of a stand, the better the expected results of reforestation and afforestation using seed collected in that stand, mainly if the aim of the operation is environmental restoring [3]. In recent years, the need of forest seeds has dramatically increased, also due to rules issued by European Community aimed at increasing and improving the forest coverage. In Italy, however, the available seeds are often of poor quality and sometimes their origin is unknown [4]: this is one of the main reason explaining the failure of many reforestation operations [5]. The need of an optimization of the forest seed production chain is therefore clear

The aim of the research was the evaluation of the effect of ordinary nursery activity on genetic variability of the processed material. The study involved 5 steps of seed production chain in Scots pine (*Pinus sylvestris* L.), namely:

- Seed stand (Olgelasca provenance, Como province)
- Seed orchard (Campogrande di Pinè, Verona province)
- Commercial seed lot collected in the seed orchard and processed by the National Forest Service factory in Dogana di Peri (Verona province)
- Nursery plantlets (two years old) located in Dogana di Peri
- Two artificial stands established using plants produced in the Dogana di Peri nursery (S. Damiano Macra and Cortemilia, both Cuneo province).

Genetic variability was estimated by means of polymorphism showed at 68 RAPD loci, amplified through PCR and electrophoretically separated on 1.5% agarose gel. DNA was extracted from young needles and embryos and, where possible, also from endosperms.
Data on genetic variability estimated for any of seed production chain steps are reported in Tab. 1. It is possible to observe a significant loss of genetic variability along the seed chain, from the seed stand to the artificial stands. It is likely that the biodiversity decrease is due to the ordinary nursery procedures, which give rise to random selection from one step of the

chain to the following. For instance, the loss of genetic variability from the seed stand to the orchard as well as from the latter to the seed lot and the nursery is probably due to the harvest of many seeds from a limited number of plants, that is those with a more abundant fructification. To the contrary, the preservation of biodiversity could be achieved harvesting seeds from a higher number of individuals. A further loss of genetic variability occurred during the establishment of the artificial stands: in this case the effect of unsuitable germplasm management procedures has to be added to natural selection. This is confirmed by the scarce success of the reforestation: many plants died in both the stands and those that survived show clear symptoms of weakening.
Since a biodiversity loss occurs already in the seed orchard (established about 25 years ago), it is suggested to increase the number of the plants with different genotypes. This should be achieved using plants grown by seeds collected in Olgelasca stand

	Mean number of alleles per locus	**Effective number of alleles per locus**	**Percentage of polymorphic loci**	**Expected heterozygosity**
Seed stand	1.86	1.78	86	0.366
Seed orchard	1.80	1.74	85	0.362
Seed lot	1.68	1.67	78	0.331
Nursery	1.54	1.62	67	0.292
Art. stand No. 1	1.43	1.58	59	0.287
Art. stand No. 2	1.39	1.52	54	0.282

Tab. 1 – Measure of genetic variability in the studied levels of Scots pine seed production chain.

On the basis of the results obtained in the present study, the Forest Seed Service of the Italian *Corpo Forestale dello Stato* decided to modify the seed harvesting procedures. Nowadays, seeds are collected form at least 30 non adjacent plants, although a higher number should be preferred.

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