SEED PROTEIN ANALYSIS OF ALHAGI (LEGUMINOSAE) SPECIES AND POPULATIONS

M. Sheidai, Z. Yazdanbakhsh & F. Bernard

Sheidai, M., Yazdanbakhsh, Z. & Bernard, F. 2002.12 30: Seed protein analysis of *Alhagi* (*Leguminosae*) species and populations. *-Iran. Journ. Bot. 9(2): 141-149.* Tehran.

Seed storage proteins were studied by using SDS-PAGE in 14 populations of two *Alhagi* species, namely *A. graecorum* Boiss. And *A. pseudalhagi* (M. B.) Desv. In total 11 protein bands were obtained among which, bands 1, 2, 4, 5, 7, 9 and 10 were common in all the taxa studied while band 3 is specific in Mehran population of *A. graecorum*. Cluster analysis of protein data revealed that such data may not be useful in differentiating the two *Alhagi* species, however they can be used in revealing the inter-population variation.

Masoud Sheidai, Zahra Yazdanbakhsh and Francoise Bernard, Department of Biology, Shahid Beheshti University, Tehran, Iran.

Key words. Alhagi, Leguminosae, seed protein, cluster analysis, Iran.

بررسی پروتئین های بذر گونه ها و جمعیت های خار شتر (Alhagi)

مسعود شیدایی ، زهرا یزدانبخش و فرانسواز برنارد

پروتئین‌های ذخیره‌ای بذر با استفاده از روش SDS-PAGE در ۱۴ جمعیت از دو گونهٔ خارشتر با نام‌های *Alhagi graecorum* Boiss. و *A. pseudalhagi* (M. B.) Desv. مطالعه شد. به طور کلی تعداد ۱۱ باند مشاهده شد که از این میان، باندهای ۱، ۲، ۴، ۵، ۷، ۹ و ۱۰ در تمامی جمعیت‌ها مشترک بودند در حالیکه باند ۳ در جمعیت همدان و باند ۱۱ در جمعیت دامغان از گونهٔ *A. pseudalhagi* منحصر بفرد بود. باند ۸ در جمعیت مهران از گونهٔ *A. graecorum* منحصر بفرد بود. تجزیهٔ خوشه ای داده های پروتئینی نشان داد که جمعیت‌های دو گونه از هم قابل تفکیک نیستند و این اطلاعات در تاکسونومی خارشتر مفید نمی‌باشند. با این حال می‌توان از اطلاعات پروتئینی در شناسایی تفاوت‌های میان جمعیتی استفاده نمود.

Introduction

The genus *Alhagi* (*Leguminosae*) comprises about 3-5 species distributed mainly in South East Europe, Turkey, Iran, Central Asia and North of Africa. *Alhagi* taxa are among important forage plants fed by camels and also used as medicinal plants for their medicinal properties. *Alhagi* species/ populations are distributed all over Iran (Fig. 1) and recently have been investigated from morphometrical and cytological point of views (Sheidai & al. 2001).

Rechinger (1984), reported 3 *Alhagi* species from Iran, namely: *A. mannifera* Desv., *A. persarum* Boiss. & Buhse and *A. pseudalhagi* (M. B.) Desv. However recent study of 88 populatiuons of *Alhagi* by Sheidai & al. (2001) by using morphometric analysis revealed the presence of only 2 *Alhagi* species in the country namely, *A. pseudalhagi* Desv. and *A. graecorum* Boiss. The same investigation revealed that karyotypic data is of no use in taxonomy of the *Alhagi* since both the species possess 2n = 16 chromosome number and there are intra-specific variations in the karyotypic details (Sheidai & al. 2001).

SDS-PAGE protein analysis has been used widly in biosystematic study of several plant species (for example: Badr 1995, Sanches-Yelamo & *al.* 1995, Sheidai & *al* 1999) as identification of seed proteins by electerophoresis has indicated that the seed protein profile is highly stable and species specific. Moreover seed protein profile is hardly affected by experimental conditions (Gray & al. 1973, Ladizinsky 1983).

Under denaturing conditions, i.e. in the presence of SDS (Sodium Dodecil Sulfate) the migration of the proteins depend on the molecular weight , since it gives a uniform negative charge to the proteins, therefore differences in detils of protein profile may indicates difference in the genes coding proteins with different molecular weights (Jhon 1989).

The present investigation considers seed storage protein analysis of *Alhagi* taxa in Iran for the first time and attempts to illustrate the use of such data in the species delimitation.

Materials and methods

Seed storage proteins were studied in 14 populations using SDS-PAGE electrophoresis (Sheidai & al. 1999). Seeds were obtained from freshly collected plants or herbarium specimens. The voucher specimens are deposited in the Central herbarium of Iran (TARI) and the Herbarium of Shahid Beheshti University (HSBU).

One hundred mg of each sample (25-50 dry seeds) was homogenized to obtain a fine powder. Proteins were extracted in a precooled mortar and pestle over ice with a 0.39 M Tris phosphate buffer (pH 8.3). The protein electrophoresis was carried out according to Sanchez-Yelamo & al. (1995), using 77 mM Tris-Hcl (pH 6.8), 4 % sodium dodecyl sulphate (SDS), 10 % 2-mercaptoethanol and 3 % glycerol and vertical slab gels of 1 mm thickness.

To estimate species/population similarity as indicated by protein electrophoresis patterns, Jaccards' and simple matching indices were determined. Each protein band was considered as a qualitative character and coded as 1 (presence) versus 0 (absence). The resulting data matrix was used for cluster analysis using single linkage and average linkage methods (Sheidai & al. 2000). Statistical methods used SYSTAT ver. 6.0.1 (1996).

Quantitative variation in proteins was determined by densitometry of bands using Corning 710 Flurimeter/ Densitometer at wave length 520 nm.

Results and discussion

The protein bands and their RM values (relative mobility), are presented in Table 1, Figs. 2 & 3. In total 11 protein bands were

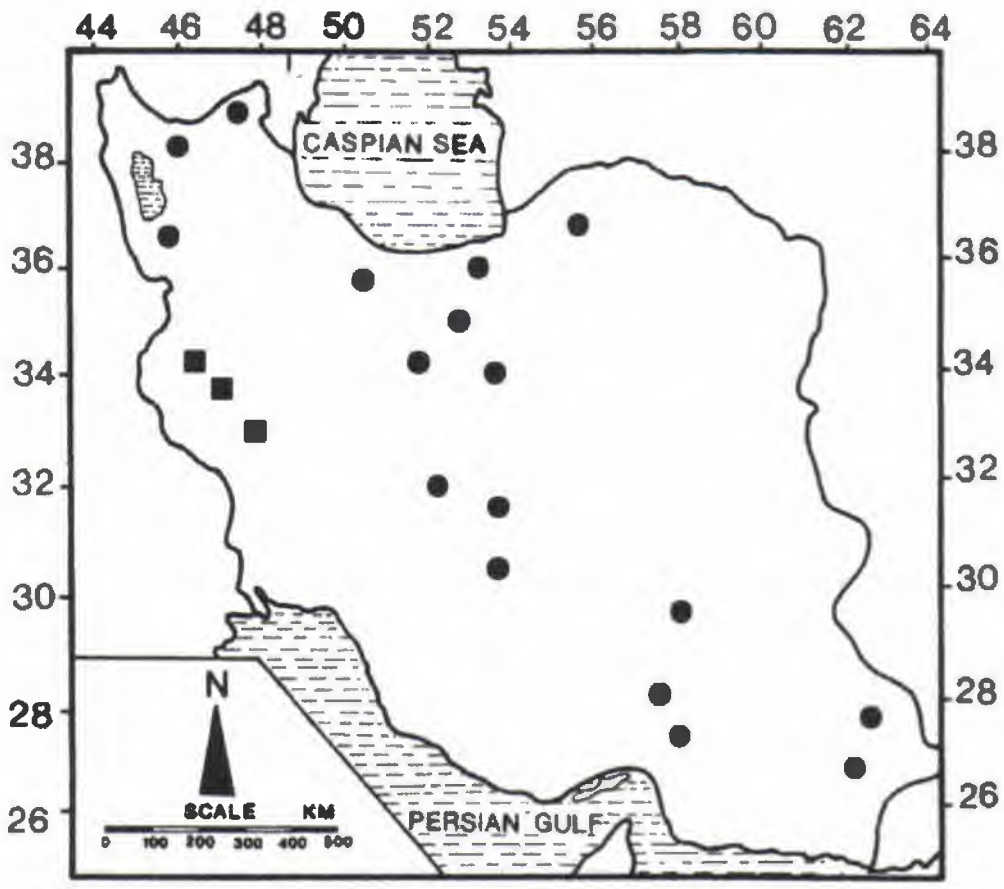


Fig. 1. Distribution of the *Alhagi* species in Iran. Circle represents *A. pseudalhagi* and rectangular stands for *A. graecorum.*

obtained from SDS-PAGE electrophoresis. Bands 1, 2, 4, 5, 7, 9 and 10 are common in all the taxa studied. Band 3 is specific in Hamedan (P10, Table 1) and band 11 in Damghan (P5) populations of *A. pseudalhagi*, while band 8 is specific in Mehran population (G2) of *A. graecorum*.

Densitometry of protein bands is presented in Fig. 4, which also indicates differences in protein profiles of the populations in each species. Quantitative diference is also evident among the common bands which is considered to be due to difference in dosage of the genes coding them (Gardiner & Forde 1992).

Table 1. The species/ populations, their protein bands and RM values.

		Species/populations code													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Band	RM	P1	P2	G1	G2	P3	P4	G3	P5	P6	P7	P8	P9	P10	G4
1	0.26	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	0.36	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	0.44	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0.46	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	0.52	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	0.56	0	0	1	1	0	0	0	1	0	0	0	0	0	0
7	0.60	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	0.70	0	0	0	1	0	0	0	0	0	0	0	0	0	0
9	0.74	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	0.80	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	0.90	0	0	0	0	0	0	0	1	0	0	0	0	0	0

Species/population code: P=*A. pseudalhagi*, P1=Mashad, P2=Mirzabaylo, P3=Abadeh, P4=Bandar Gaz, P5= Damghan, P6=Urmieh, P7=Tabriz, P8=Gorgan, P9=Bandar Torkaman, P10=Hamedan. G=*A. graecorum*, G1=Sarepolezahab, G2=Mehran, G3=Ghasreshirin, G4=Unknown.

Several studies indicate that the protein data is not useful in the taxonomy of *Leguminosae* at the species level (Polhill & Raven 1981), however some others indicate the use of such data in taxonomic treatment and elucidating the species interrelationships (Badr 1995, Sheidai & al. 1999).

Different methods of cluster analysis of protein data using both Jaccard as well as simple matching coefficients produced similar results therefore only UPGMA cluster analysis based on Jaccard index is presented in Fig. 5 (Table 2). Although Sarepolezahab (G1) and Mehran (G2) populations of *A. graecorum* are grouped together, two populations of G4 and Ghasreshirin (G3) show similarity to *A. pseudalhagi* populations and are placed in one cluster. The same is true for Damghan population (P5) of *A. pseudalhagi* which is separated from the other populations. Therefore it seems that protein data can not separate the two *Alhagi* spp. However such data may be used to indicate inter-population differences as Damghan (P5) and Hamedan (P10) populations of *A. pseudalhagi* stand in separate clusters far from the other populations of *A. pseudalhagi*.

As stated earlier, cytological data could not differentiate the two species of *Alhagi*. Both species possessed 2n = 16 chromosome number and their karyotypic features including size of the chromosomes, number of SAT-chromosomes and karyotypic formulae differ among different populations and may not be used for the species delimitation (Sheidai & *al.* 2001). The same statement is true for protein characteristics of *Alhagi* species including number of protein bands as well as quantitative features of the common bands. These may indicate that differences in karyotypic as well as protein features of *Alhagi* populations are genotypic adaptation to their environmental conditions. Therefore, for the time being morphological characters are the only useful discriminating criteria for taxonomic treatment of *Alhagi*.

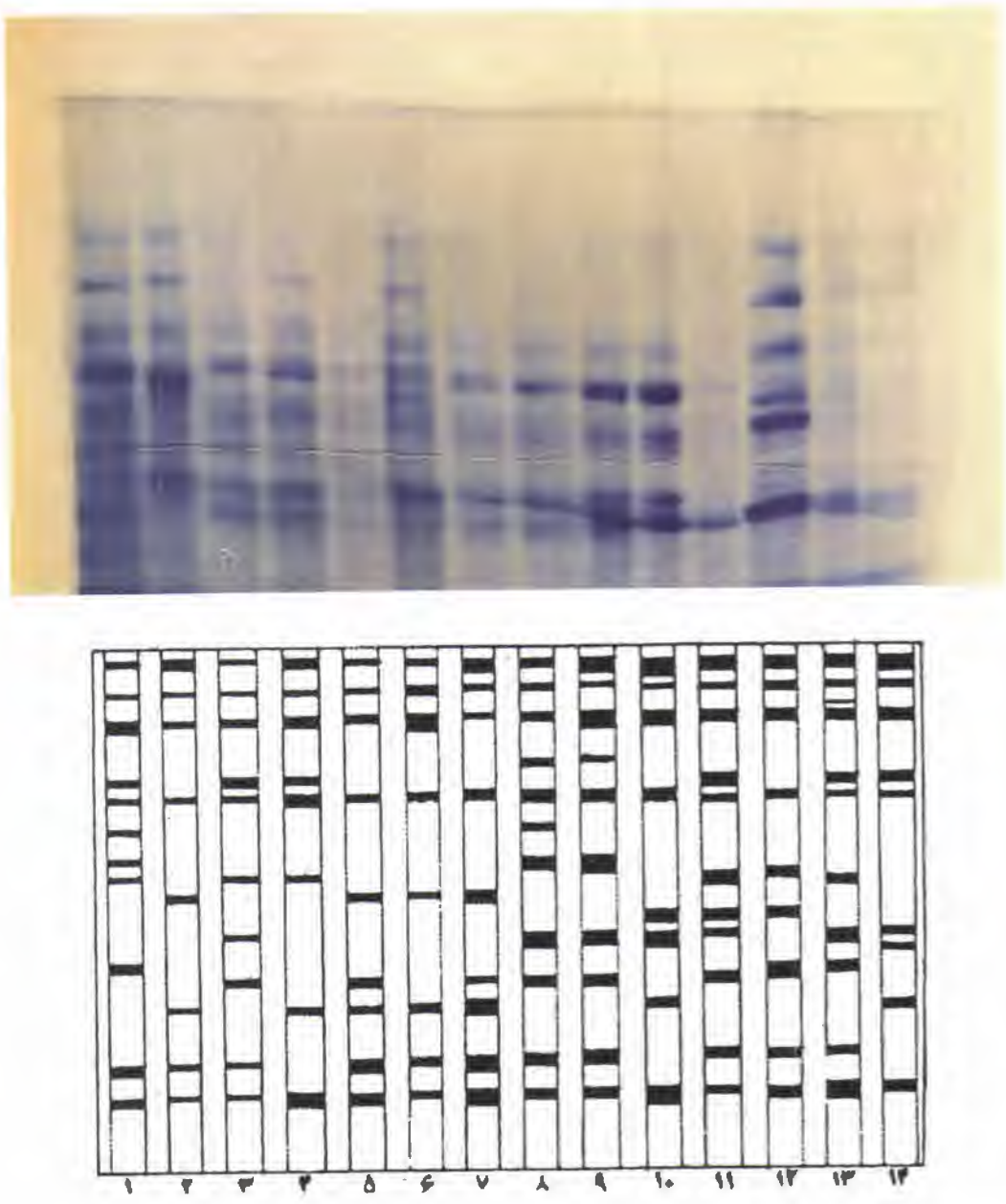
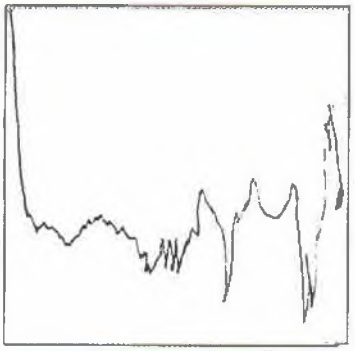
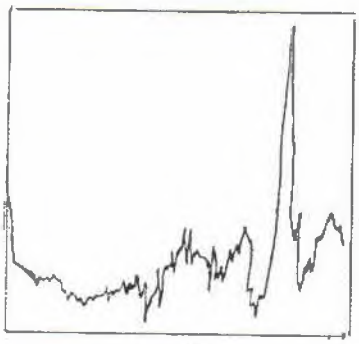


Fig. 2 & 3. SDS-PAGE protein bands in *Alhagi* species/ populations. Species/populations from left to right are 1-14 of table 1.



1



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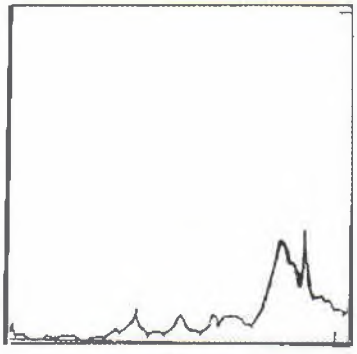

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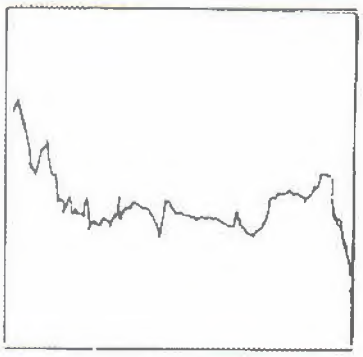

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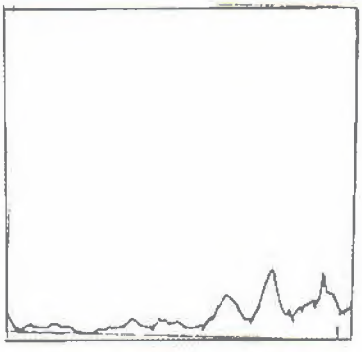

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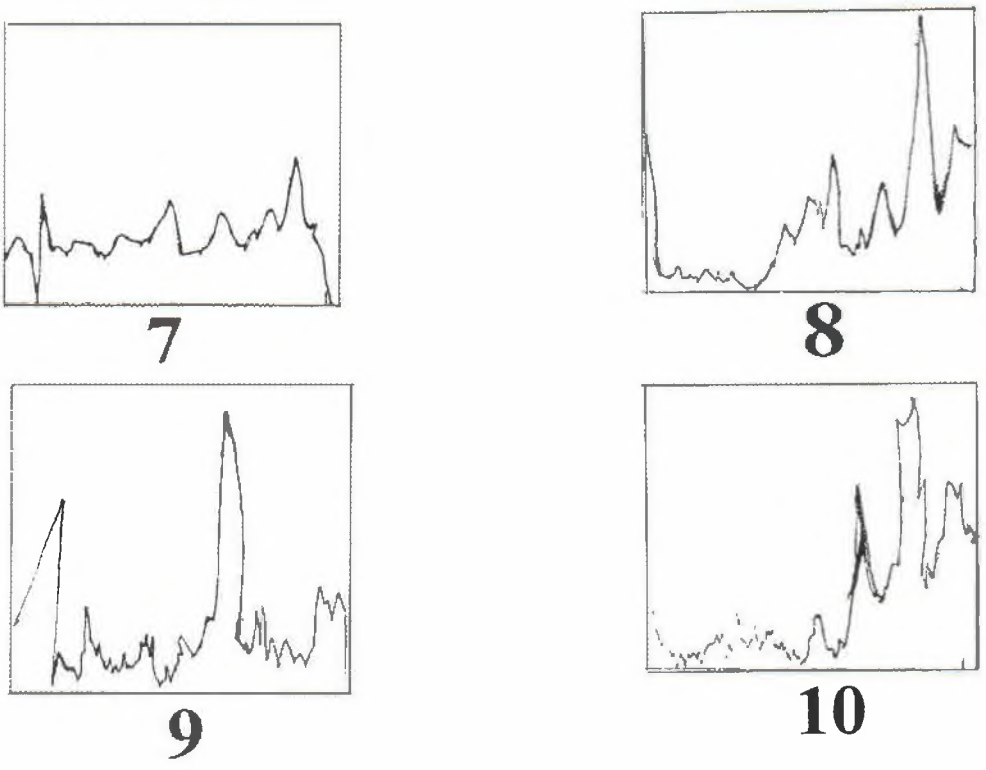

Fig. 4. Representative densitometry curves of *Alhagi* species and populations. Populations 3, 4 & 6 belong to *A. graecorum* while the others belong to *A. pseudoalhagi.*

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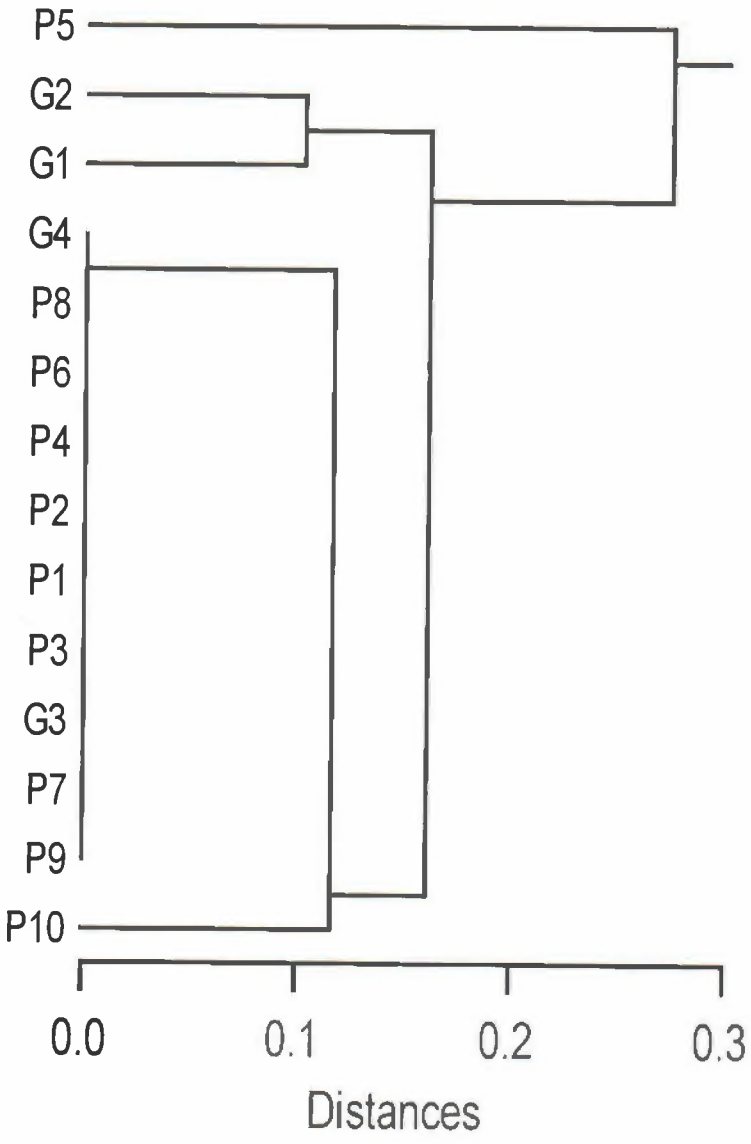

Fig. 5. UPGMA cluster analysis of protein data. Species/ populations code as in Table 1.

Table 2. Jaccard similarity index among *Alhagi* species and populations. Numbers 1-14 is according to Table 1.

Sp	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1		1.000	0.875	0.778	1.000	1.000	1.000	0.667	1.000	1.000	1.000	1.000	0875	1.000
2	1.000		0.875	0.778	1.000	1.000	1.000	0.667	1.000	1.000	1.000	1.000	0.875	1.000
3	0.875	0.875		0.889	0.875	0.875	0.875	0.778	0.875	0.875	0.875	0.875	0.778	0.875
4	0.778	0.778	0.889		0.778	0.778	0.778	0.700	0.778	0.778	0.778	0.778	0.700	0.778
5	1.000	1.000	0.875	0.778		1.000	1.000	0.667	1.000	1.000	1.000	1.000	0.875	1.000
6	1.000	1.000	0.875	0.778	1.000		1.000	0.667	1.000	1.000	1.000	1.000	0.875	1.000
7	1.000	1.000	0.875	0.778	1.000	1.000		0.667	1.000	1.000	1.000	1.000	0.875	1.000
8	0.667	0.667	0.778	0.700	0.667	0.667	0.667		0.667	0.667	0.667	0.667	0.600	0.667
9	1.000	1.000	0.875	0.778	1.000	1.000	1.000	0.667		1.000	1.000	1.000	0.875	1.000
10	1.000	1.000	0.875	0.778	1.000	1.000	1.000	0.667	1.000		1.000	1.000	0.875	1.000
11	1.000	1.000	0.875	0.778	1.000	1.000	1.000	0.667	1.000	1.000		1.000	0.875	1.000
12	1.000	1.000	0.875	0.778	1.000	1.000	1.000	0.667	1.000	1.000	1.000		0.875	1.000
13	0.875	0.875	0.778	0.700	0.875	0.875	0.875	0.600	0.875	0.875	0.875	0.875		0.875
14	1.000	1.000	0.875	0.778	1.000	1.000	1.000	0.667	1.000	1.000	1.000	1.000	0.875	

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