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Comparative evaluation of chamomile (*Matricaria recutita* L.) populations from different origin

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Summary: In our research project under cultivation we examined 4 cultivars ('Soroksári 40', 'Lutea', 'Goral', 'Bona') and 28 wild populations of chamomile assuring all of them the same environmental conditions. Plant height, flower-diameter, essential-oil content, and the main terpenoid and flavonoid composition were analysed. The aim of our study was to establish the genetic background of breeding a new cultivar as well as encircle those natural habitats that give chamomile drug with the best quality.

In the case of plant height populations from the Great Hungarian Plain were lower than plants from Transdanubia and the control cultivars. Between the wild and the standard individuals we found significant differences with relevance to the flower-diameter. With regard to the essential oil content the populations were very heterogeneous even those, which came from the same habitat. Populations form Transdanubia and Nagyiván reached the essential oil level of the cultivars (0.721–0.931 g/100g), and 75% of the examined plants exceeded the minimum requirement of the PhHg VIII.

According to the essential oil composition our previous statement was confirmed that in the populations of Transdanubia and Northern part of Danube–Tisza Mid Region the main component is bisabolol-oxide A (30–41.2%), while plants native to the territory east of the river Tisza are mainly characterised by α-bisabolol (32.3–48.4). In some samples the ratio of bisabolol-oxide B was more than 10%. The chamazulene content was higher in the cultivars selected to this component (above 20%), than in the wild populations (varied between 1.22 and 17.2%). Populations originated from the central part of Hortobágy region had extremely high apigenin content (10–13 mg/g), but in the case of chlorogenic acid, hyperoside and quercitrin we did not find any differences affected by the origin.

Key words: Chamomilla recutita, Hungary, plant-height, flower-diameter, essential oil, chamazulene, α-bisabolol, bisabolol-oxide, apigenin, flavonoid

Introduction

Chamomile (*Matricaria chamomilla* L., syn.: *Matricaria recutita* L. or *Chamomilla recutita* L.) is traditionally used in Hungary and it is a well-known national product in the export market. The drug yield is 150–250 t per year, so chamomile is one of the most important 10 collected and cultivated medicinal plants in Hungary. Due to the unique ecological conditions of the wild habitats, the drug – *Chamomillae anthodium* – known as a real „Hungaricum" is famous for its excellent quality.

Chamomile is very common in Hungary, and the place of the origin could affect the plant-height, chemical composition and drug yield. In the west part of Transdanubia and also in Bakony hills it occurs dispersedly, mainly in ruderal fields or sometimes in arables. In these habitats the plants are usually bigger and more branched. In the Great Hungarian Plain it is also found in ruderal and agricultural territories, but most of the populations are living on alkali soils, where the plants are smaller and less branched.

The drug of the plant is the flower (*Chamomillae anthodium*) and the essential oil (*Aetheroleum chamomillae*). Among its active agents we can find volatile and non volatile components.

The flowers contain 0.4–1.2% essential oil, wherein the main components are chamazulene and bisaboloids. Its blue colour is due to the chamazulene, that is a secondary product of hydrodistillation and it is a result of proazulenes' (such as *matricin*) transformation (*Svábné*, 2000).

According to earlier surveys in the populations of Transdanubia the number of the individuals, that are free from proazulenes, is minimal. These plants are usually characterised by high proazuelen content. However, on the alkali soils of the territory east of the river Tisza, the plants contain less proazulenes, and the number of the proazulene-free individuals is higher (*Máthé Prisztер*, 1979). But this statement could not been confirmed definitely by either the research project of *Sztefanov* (2003) or our prevoius work (*Gosztola* et al., 2005).

Other components of the essential oil such as α-bisabolol, that is a sesquiterpene alcohol, and its oxides such as bisabolol- and bisabolon-oxide are also have an important pharmaceutical activity as well as farnesene, that has juvenil hormonal effect (*Petri*, 1982).

Among its non volatile components the most important are flavonoids, coumarins, and pectine-like mucilages. The most well-known flavonoids are apigenin, chlorogenic acid, hyperoside, quercitrin and their glycosides, that have spasmolitye effect (*Máthé Priszter*, 1979). Sztefanov et. al made an experiment (2002, 2003) on chamomile first with relevance to its non volatile components. According to their findings there is no connection between the plant material origin and the level of the non volatile components.

Breeding work of chamomile in Hungary was started in the 1960's. As a result of it today we have two Hungarian cultivars: *'Budakalászi 2'* and *'Soroksári 40'*. Since 1970, the year when they were officially registrated, new cultivars haven't been announced. Nowadays, in cultivation the peresence of the above mentioned Hungarian cultivars is not significant, that can be explained by different reasons. One problem is the lack of the maintaining-breeding work of the cultivars. The other is the plant material coming from abroad, which is more competitive than the domestic products. Because of production on behalf of foreign companies Hungarian cultivars are going to disappear from cultivation.

Our aim was to continue chamomile selection started before at the department making the possibility to define those Hungarian natural habitats and populations which are suitable to product high quality drug. The selection of diverse genotypes is also making the possibility of breeding new cultivars, specialized for different kind of pharmaceutical usage.

Material and Method

In May, 2004 many wild chamomile populations were sampled and got involved into the research started by Sztefanov *et al.* (2003) earlier.

In every wild population we collected cribratum for propagation. In 2005, in the Research Station of the Department, Soroksár, we sowed the collected cribratum assuring the same environmental conditions for the plants. As a controll four cultivars – one Hungarian 'Soroksári 40' (K/29) (diploid), and three Slovak cultivars: 'Lutea' (K/30), 'Goral' (K/31) (tetraploids), and 'Bona' (K/32) (diploid) were used. The examined chamomile populations can be seen in *Figure 1.* and *Table* 1. The propagation was carried out in greenhouse, the seedlings were transplanted into open field on 7, April by using plant density of 40×20 cm, arranged in 2,2×1,6 m^2 plots without replication.

Table 1. The examined chamomile populations

1	K/1	Hortobágy National Park, field 1
	K/2	Hortobágy National Park, field 2
	K/3	Hortobágy National Park, field 3
	K/4	Hortobágy National Park, field 4
2	K/5	Polgár (Hajdú-Bihar County)
3	K/6	Beleg (Somogy County)
	K/7	Kisbajom (Somogy County)
	K/8	Szabás (Somogy County)
4	K/9	Nádudvar, field 1
	K/10	Nádudvar, field 2
5	K/11	Nagyiván, field 1
	K/12	Nagyiván, field 2
	K/13	Nagyiván, field 3
	K/14	Nagyiván, field 4
	K/15	Nagyiván, field 5
	K/16	Nagyiván, field 6
	K/17	Nagyiván, field 7
	K/18	Nagyiván, field 8
6	K/19	village Hortobágy, field 1
	K/20	village Hortobágy, field 2
	K/21	village Hortobágy, field 3
7	K/22	Borsodivánka, field 1
	K/23	Borsodivánka, field 2
	K/24	Borsodivánka, field 3
8	K/25	Tiszafüred, field 1
	K/26	Tiszafüred, field 2
9	K/27	Kunmadaras
10	K/28	Kócsújfalu
cultivar	K/29	'Soroksári 40
cultivar	K/30	'Lutea'
cultivar	K/31	'Goral'
cultivar	K/32	'Bona'

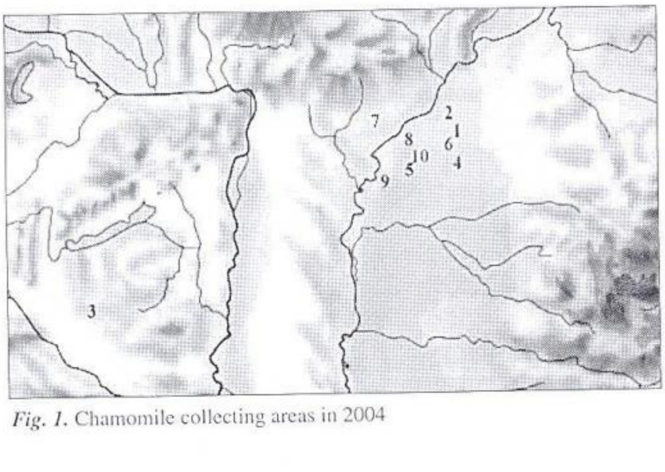

Fig. 1. Chamomile collecting areas in 2004

The morphological observations were carried out on 20, randomly chosen individuals. In each population we examined the plant height – whole sprout length – and the diameter of 5 flowers at the end of the primary and secondary sprouts in full blossoming. In the case of chemical analysis essential-oil content and its composition as well as the amount of the main flavonoids were examined in every population. We repeated our measurements three times. Hydrodistillation, determination of essential oil content by GC and thin-layer chromatography (TLC) for flavonoid analysis were carried out according to Sztefanov *et al.* (2003).

Results and discussion

Morphological characteristics

The results of plant height are summarized in *Figure 2*. The cultivars used as control were significantly higher than the examined wild populations. Cultivar 'Soroksári 40' (K/29) was the highests (34.5 cm), but also the Slovak cultivars – 'Bona' and 'Lutea' – were characterised by good results. However between diploid and tetraploid cultivars we did not find great differences. Populations from Transdanubia (K/6, K/7, K/8) also exceeded the average level of plant height (22.2 cm) as well as the populations from Tiszafüred (K/25, K/26) and Hortobágy village (K/19, K/20, K/21). Plants from Hortobágy National Park (K/1, K/2, K/3, K/4) were the smallest with an average plant-height of 14.3-18.1 cm, and populations of Nagyiván (K/11, K/12, K/13, K/14, K/15, K/16) were also characterised by low results. These findings are in agreement with our previous statements (*Gosztola* et al., 2005), that plants from Transdanubia are significantly higher than the populations of Hortobágy, and this kind of difference can be also observed under cultivation.

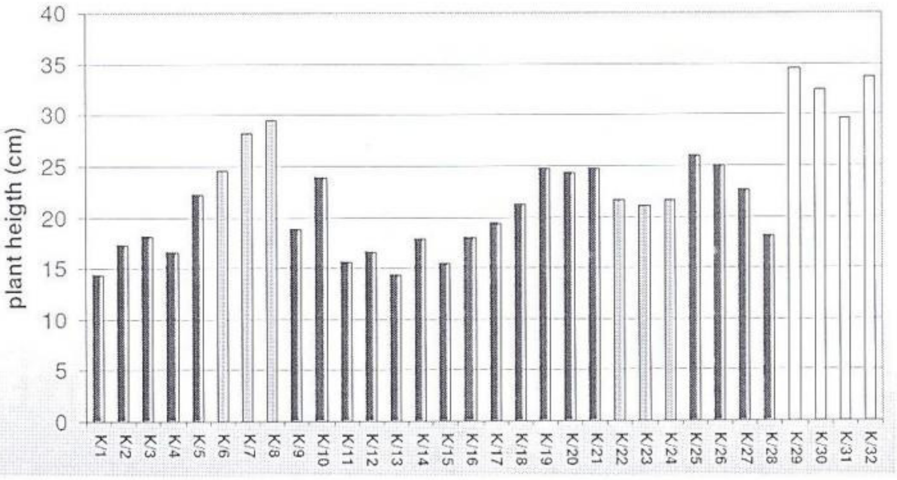

Fig. 2. Plant heigth of the examined chamomile populations under cultivation in 2005, Soroksár (SD5%=2.98 cm)

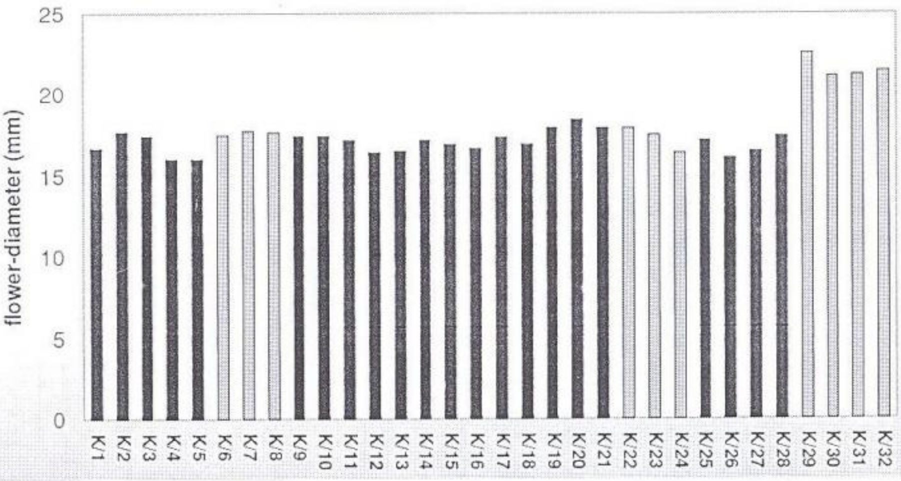

Fig. 3. Flower-diameter of the examined chamomile populations under cultivation in 2005, Soroksár (SD5%=1.47 mm)

In the case of flower-diameter we came to the conclusion that the cultivars have significantly bigger flowers than the wild populations (*Figure 3*). Cultivar 'Soroksári 40' showed the best results again (22.6 mm). The other cultivars also exceeded the average flower-diameter (20 mm), while the wild populations had smaller flowers. Among the wild populations plants from Transdanubia and Hortobágy village (K/19, K/20, K/21) were characterised by relatively good results (17.9-18.4). The smallest flowers were found in the habitats signed K/4, K/5, K/23 and K/26 where the flower diameter was just approximately 16 mm.

Chemical composition

With regard to the essential oil content, the populations were very heterogeneous (*Figure 4*). The highest essential oil content (0.93 g/100g) was measured in the Slovak tetraploid cultivar 'Goral'. According to the general chamomile characterisation of Lutomski and Czabajska (1993) this value is in the low section of the interval 0.8–1.5%. Second highest result was found in the population of Beleg (K/6), that exceeded the level of the three remained cultivars ('Soroksári 40', 'Lutea', 'Bona'). In 2005 the cultivar 'Soroksári 40' did not reach the essential oil content ascertained in its cutivar description (0.8%). Other populations from Transdanubia (K/7 and K/8) and plants from Nagyiván were also characterised by good results (0.6%). It was very interesting, that the populations of Nagyiván (K/11, K/12, K/13, K/14, K/16, K/17, K/18) – that were mostly a part of one great population – were significantly different from each other with relevance to their essential oil content. This fact indicates that in the case of essential oil accumulation even inside one population great differences could be observed.

The lowest essential oil content was measured in the populations signed K/10, K/11, K/19, K/21, K/23, K/25, K/26, where the results did not meet the minimum requirement (4 ml/kg) of the Ph.Hg.VIII. Population of Tiszafüred (K/26) produced only 0.2% essential oil.

These results are in agreement with our previous findings (*Gosztola* et al., 2005) that chamomile populations from Transdanubia both in their natural habitat and under cultivation are characterised by higher essential oil content than plants from Hortobágy region.

In the case of the essential oil composition the main components – chamazulene, bisabolol-oxide A, bisabolol-oxide B, α-bisabolol and farnesene – were found in every population but their amount was different (*Table 2*). According to *Schilcher* (1987) the populations can be divided into three groups. Chemotype"A": the main component is bisabolol-oxide A (K/6, K/7, K/8 – from Transdanubia, K/22, K/23, K/24 – from Borsodivánka and territory west of the river Tisza and the cultivar 'Soroksári 40'). In these populations the ratio of bisabolol-oxide A varied between 30–41%. Chemotype "C": the main component is α-bisabolol (32–48%) (populations east of the river Tisza, cultivars 'Lutea' and 'Bona'). Chemotype "D": the amount of bisabolol-oxide A, B and α-bisabolol is approximately equal (K/20, K/25, K/27 and the cultivar 'Goral'). The differences in the essential oil composition can be demonstarted by Cluster analysis (*Figure 5*). First populations belong-ing to chemotype "A" differentiate, then plants from chemotype "C" and "D".

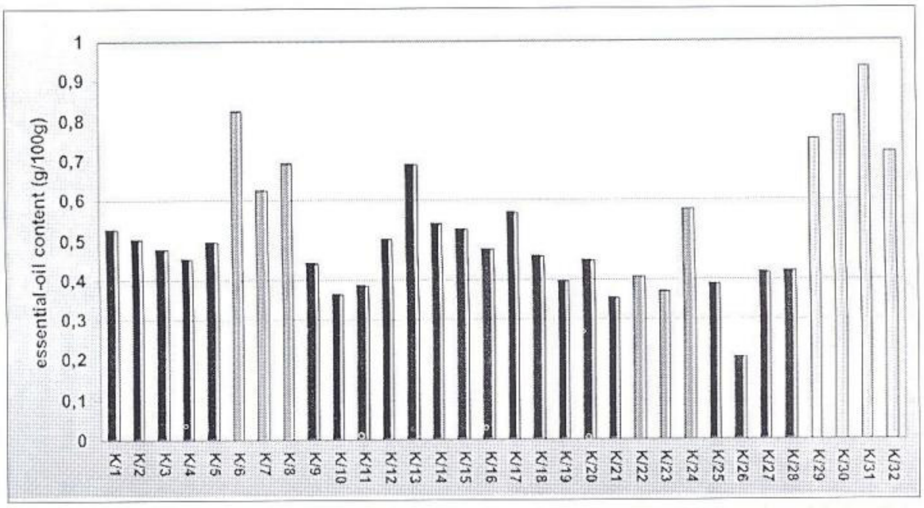

Fig. 4. Essential-oil content of the examined chamomile populations under cultivation in 2005, Soroksár (SD5%=0.11 g/100 g)

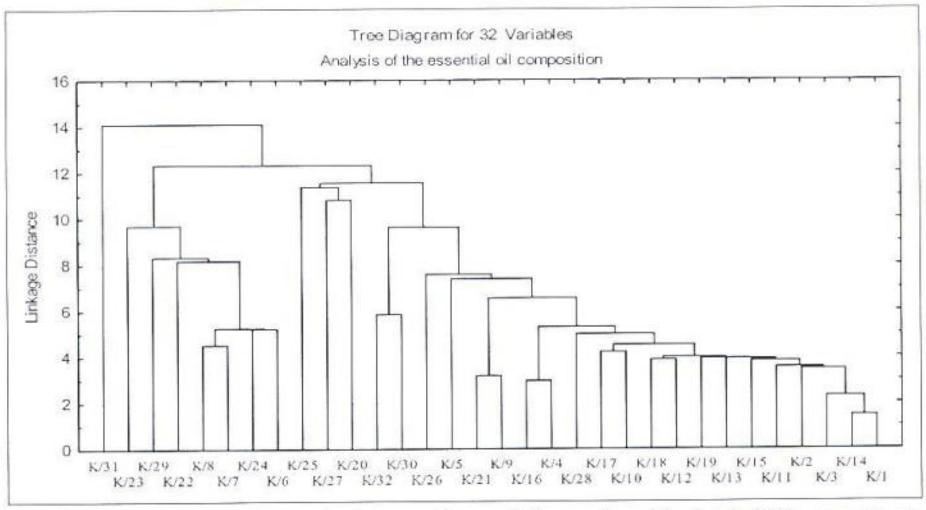

Fig. 5. Relations between the examined chamomile populations under cultivation in 2005, accroding to Cluster-analysis, depending on the essential oil composition

Place of origin had an influence on the essential oil composition. While the populations of Somogy region and north part of Danube Tisza Midregion are belonging to bisabolol-oxide A chemotype, in the essential oil of plants from east of the river Tisza the main component is α-bisabolol. In the case of the three remained populations the amount of bisabolol-oxide A, B and α-bisabolol was almost the same. *Sztefanov* et al. (2003) got the same result with relevance to their 12 examined populations.

In the case of chamazulene content it is ascertained that the cultivars are richer in chamazulene (21–24.5%) than the wild populations, in which only K/7, K/8, K/11 and K/14 exceeded the average chamazulene content (13–17.2%).

The apigenin content of the populations was significantly higher than the results of Sztefanov et al., 2003 (1.316-2.800 mg/g). From this point of view plants from Hortobágy National Park (K/2, K/3, K/4), Nagyiván and Hortobágy village (K/8, K/13, K/14, K/15, K/16, K/17, K/18, K/19, K/21, K/22, K/23) and the cultivar 'Bona' were characterised by the best results (10.1–14.1 mg/g) (Table 3.). All of the populations met the minimum requirement of the Ph.Hg. VIII (0.25%).

With regard to other flavonoids the highest chlorogenic acid level (0.87–1.57 mg/g) was found in the populations signed K/17, K/18, K/21, K/22 and K/27. In the case of hyperoside K/2, K/16, K/17, K/18, K/20, K/21, and K/23 showed the best results (12.2-14.4 mg/g), while cultivars 'Goral', 'Bona' and populations K/1, K/8, K/21, K/26, K/28 were characterised by relaively high quercitrin content. However, referring to these components we did not find any evidence to the effect of the origin.

Acknowledgement

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Table 2. Variation in main essential oil components in 2005 (%)

	farnesene (%)	bisabolol-oxid B (%)	alfa-bisabolol (%)	chamazulene (%)	bisabolol-oxid A (%)
K/1	4.2	8.2	40.7	13.5	2.3
K/2	3.8	10.0	43.9	11.0	3.1
K/3	2.9	8.1	39.8	11.9	2.7
K/4	3.2	7.5	46.6	7.6	0.2
K/5	4.6	9.9	32.3	12.3	5.6
K/6	2.9	11.2	8.4	12.3	41.2
K/7	3.0	10.3	8.0	13.3	34.4
K/8	2.8	9.5	6.7	17.2	36.1
K/9	4.52	6.12	45.49	1.44	0
K/10	4.28	12.96	35.58	6.35	0
K/11	4.61	7.41	41.95	13.42	5.5
K/12	4.5	10.77	44.06	7.73	5.79
K/13	4.89	9.2	40.21	9.88	5.12
K/14	3.9	9.01	41.66	13.08	1.81
K/15	4.2	6.56	42.87	9.81	2.92
K/16	4.8	6.53	48.35	8.42	1.43
K/17	5.29	13.46	36.98	8.77	2.94
K/18	4.8	12.28	41.14	7.95	3.79
K/19	6.78	8.56	38.94	11.33	1.42
K/20	6.12	18.27	26.1	7.83	7.23
K/21	3.6	5.42	48.44	1.22	0
K/22	5.15	10.86	6.43	7.28	29.59
K/23	3.88	6.58	12.46	11.99	25.65
K/24	4.55	9.84	6.73	9.67	37.59
K/25	4.35	12.03	17.09	12.19	15.63
K/26	4.03	3.47	34.43	12.58	0
K/27	5.25	21.73	17.86	6.9	13.18
K/28	5.2	15.28	39.98	4.35	2.62
K/29	1.7	4.1	4.7	23.0	35.0
K/30	3.16	2.48	37.31	24.5	1.69
K/31	3.01	9.39	19.31	24.41	9.65
K/32	3.95	2.34	41.92	20.99	1.59

Table 3. Variation in main flavonoid components in 2005 (mg/g)

	apigenin (apigenin-7-glükozid) (mg/g)	chlorogenic acid (mg/g)	hyperoside (mg/g)	quercitrin (mg/g)
K/1	8.634	0.443	11.106	0.721
K/2	10.356	0.534	12.706	0.308
K/3	10.142	0.434	11.849	0.521
K/4	10.052	0.387	11.983	0.283
K/5	8.740	0.729	10.729	0.467
K/6	9.969	0.804	10.627	0.530
K/7	9.820	0.711	10.893	0.679
K/8	10.104	0.000	11.486	0.701
K/9	9.243	0.260	10.960	0.438
K/10	9.122	0.710	9.458	0.193
K/11	9.381	0.372	9.483	0.376
K/12	9.608	0.598	8.984	0.157
K/13	11.144	0.594	8.731	0.416
K/14	11.509	0.701	9.259	0.586
K/15	10.993	0.782	9.401	0.507
K/16	11.151	0.692	12.487	0.276
K/17	12.950	0.928	12.613	0.390
K/18	12.466	1.167	12.184	0.261
K/19	11.912	0.703	11.341	0.457
K/20	7.911	0.649	13.001	0.447
K/21	10.077	1.136	14.040	0.654
K/22	10.661	0.864	12.770	0.128
K/23	10.909	0.374	13.451	0.097
K/24	6.168	0.397	7.990	0.179
K/25	7.581	0.223	8.057	0.094
K/26	8.092	0.456	9.338	0.833
K/27	7.616	1.569	8.500	0.446
K/28	6.498	0.243	7.957	0.654
K/29	8.057	0.093	7.810	0.380
K/30	8.076	0.619	8.427	0.386
K/31	8.327	0.222	8.279	1.598
K/32	14.143	0.076	10.790	1.432

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