

Chromosome numbers of the *Medicago sativa* complex in Turkey

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Turkish representatives of the *Medicago sativa* complex comprise three basic morphological classes distinguishable by the following: (1) purple flowers, coiled pods; (2) yellow flowers, uncoiled pods; (3) intermediates and recombinants between 1 and 2. Chromosome determinations were made for 329 plants representing 35 cultivated (alfalfa) and 87 wild populations. All cultivars collected fell into class 1 and were tetraploid ($2n = 4x = 32$). Wild plants representative of all three morphological classes were found at both the diploid ($2n = 2x = 16$) and tetraploid ($2n = 4x = 32$) level, and examples of hybrid zones between classes 1 and 2 were common at both levels. Most populations appeared to contain plants at the same ploidy level, but a few contained a mixture of both diploids and tetraploids, and at several locations diploid and tetraploid populations grew adjacent to each other. Tetraploids were widespread across Turkey whereas diploids were confined to the northeast region.

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Les représentants turcs du complexe du *Medicago sativa* se répartissent en trois types morphologiques fondamentaux: (1) des plantes à fleurs pourpres et à gousses spiralées, (2) des plantes à fleurs jaunes et à gousses non spiralées et (3) des plantes intermédiaires et des recombinants entre les types 1 et 2. Les auteurs ont déterminé le nombre chromosomique de 329 plantes représentant 35 populations cultivées et 87 populations sauvages. Tous les cultivars appartiennent au type 1 et sont tétraploïdes ($2n = 4x = 32$). Parmi les plantes sauvages, les trois types morphologiques sont représentés, aussi bien au niveau diploïde ($2n = 2x = 16$) qu'au niveau tétraploïde ($2n = 4x = 32$), et l'on trouve des zones hybrides entre les types 1 et 2 aux deux niveaux de ploïdie. La plupart des populations semblent être composées d'individus ayant un même nombre chromosomique, mais quelques populations comprennent un mélange de diploïdes et de tétraploïdes et, dans plusieurs localités, les diploïdes et les tétraploïdes sont adjacents l'un à l'autre. Les tétraploïdes sont répandus dans toute la Turquie, tandis que les diploïdes sont confinés à la région nord-est.

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Introduction

The name *Medicago sativa* L. has been variously applied to some or all of a diverse set of wild variants, in addition to the cultigen alfalfa, the world's most important forage domesticate. This "species" is particularly heteromorphic in the US-SR, and Russian taxonomists have tended to recognize numerous variants as distinct species (Vassilczenko 1949; Sinkaya 1950; Grossheim 1971; Lubenets 1972). Ivanov (1980) gives a detailed interpretation of the cytology and geography of 11 "species," which may all be referable to the *M. sativa* complex, and whose status requires clarification. The present analysis of the complex is confined to Turkey where, as indicated by Davis (1970), there is a critical need to clarify the relationships between ploidy, morphology, and nomenclature. This contribution focuses on ploidy distribution within Turkey; work is in progress on the morphological relationships between the ploidy level variants reported here.

Turkey was chosen for an intensive study of the *M. sativa* complex because it provides the most accessible area to Western scientists for the examination of wild and primitive cultivated plants related to alfalfa. It is believed that the site of origin of alfalfa is in the foothills and mountain valleys of Armenia, eastern Anatolia, Iran, Afghanistan, central Asia, Jamma, and Kashmir. Excellent accounts of alfalfa evolution are found in Lesins (1976) and Lesins and Lesins (1979). Modern cultivars of alfalfa are rarely grown in Turkey, where the predominant alfalfas include primitive land races commonly labelled "native types," and an ancient indigenous kind of

cultivar called "Kayseri alfalfa" (for a fuller description of Turkish alfalfa see Alinoğlu et al. 1972; Christiansen-Weniger and Tarman 1939; Small 1981, 1982).

Davis' treatment (1970) of the Turkish members of the *M. sativa* complex recognizes three basic taxa. Purple-flowered plants with coiled pods are placed in *M. sativa*, this comprising two intergrading variants, subspecies *sativa* and subspecies *caerulea* (Less. ex Ledeb.) Schmalh. The latter has smaller flowers and fruits than the former, and a diploid ($2n = 16$) chromosome complement, whereas the former has " $2n = 32; 16?$ " (Davis 1970, p. 488). Yellow-flowered plants with uncoiled pods are placed in *M. falcata* L., for which Davis stated that both diploid and tetraploid populations had been reported (but apparently not diploids in Turkey). Finally, more or less stabilized hybrid swarms between the two "species" are placed in *M. × varia* Martyn, some collections of which have been classified under different species names, and for which outside Turkey both diploids and tetraploids have been recorded.

Because it seems apparent that there is extensive morphological intergradation between the variants, subspecific nomenclature is employed in this paper, viz. *M. sativa* subsp. *sativa*, subsp. *caerulea*, subsp. *× varia* (Martyn) Arcangeli, and subsp. *falcata* (L.) Arcangeli (as employed by Gunn et al. 1978; cf. Small and Brookes 1984). Table 1 summarizes current nomenclature. It should be noted that at present a moderately reliable macroscopic means of separating diploid and tetraploid purple-flowered plants with coiled pods is available (Davis 1970), but not for yellow-flowered plants with uncoiled

TABLE 1. Subspecific nomenclature applied to morphological-chromosomal variants of *Medicago sativa* L. s.l.

Ploidy	Prominent morphological features		
	Purple flowers, coiled pods	Intermediates	Yellow flowers, uncoiled pods
Tetraploids ($2n = 4x = 32$)	<i>sativa</i>	\times <i>varia</i>	<i>falcata</i>
Diploids ($2n = 2x = 16$)	<i>caerulea</i>	\times <i>varia</i>	<i>falcata</i>

Pods or intergradients (see, for example, Lesins and Lesins 1979). However, pollen diameter may be used with appreciable success to discriminate diploid and tetraploid plants (Small 1983).

Diploid and tetraploid chromosome counts have been reported under a variety of species names which pertain to the *M. sativa* complex (Fedorov 1969), but the taxonomy of the group is presently too poorly understood to interpret many of the reports. The best analysis of cytological variation in *Medicago* currently available is that of Lesins and Lesins (1979), which reduces most of the names of the complex to synonymy with the variants recognized in this paper, and additionally accepts some variations for which there is no reliable evidence of occurrence in Turkey (*M. glomerata* Balbis, *M. glutinosa* Bieb.).

Materials and methods

An expedition to collect seeds of wild and domesticated variants of *M. sativa* was conducted in Turkey from July 15 to August 13, 1981, under the auspices of the Regional Agricultural Research Institute at Menemen, Turkey. The seed collections were divided among that institute, Agriculture Canada, and the United States Department of Agriculture (represented on the expedition by Drs. J. H. Elgin, Jr., and R. H. Ratcliffe). The route followed traversed the length of Turkey through its central portion and additionally emphasized northeastern Turkey, which is notably mountainous and climatically and floristically distinct. A full account of the expedition may be found in Small (1981).

Meiotic and (or) somatic chromosome number determinations were made for the collected material of the *M. sativa* complex. Thirty-three collections of flower buds, representing 24 wild populations, were made in the field in Turkey and preserved in 3:1 ethanol-acetic acid, and chromosome number determinations were made in Ottawa using the aceto-carmine squash technique. In many cases these field collections were from populations with plants too immature for seed acquisition. For 35 wild populations with viable seeds (including 3 populations which had floral buds collected in the field in Turkey) and 34 cultivated populations, plants were established in a greenhouse in Ottawa. Subsequently, floral buds were fixed in modified Carnoy's fluid (6:3:1 ethanol-chloroform-acetic acid), and meiosis in microsporocytes was examined following staining by Snow's (1963) modification of the aceto-carmine squash technique. In one case (collection 20) floral buds had not formed after almost 1 year, and somatic chromosome determinations were made using root tip squashes in aceto-orcin following pretreatment in 0.027% colchicine (cf. Tjio and Levan 1950). Somatic chromosome determinations were also made for 36 collections being grown for seed increase at Beltsville, Maryland. For these, root tips were pretreated with a saturated solution of paradichlorobenzene for 3.5 h, fixed in Carnoy's fluid, hydrolyzed for 20 min in 1 N HCl at 60°C, placed in Feulgen's stain for 1 h, and squashed in aceto-carmine. Because it was thought that the cultivated collections were likely to be uniform, usually only one plant was examined cytologically. By contrast, where available about four

plants of the wild populations were examined. Herbarium vouchers were preserved of each of the 185 individual plants from which chromosome counts were made at Ottawa. For almost all of the populations, vouchers were collected in the field in Turkey during the expedition there. The vouchers are in the herbarium of Agriculture Canada, Ottawa.

Herbarium vouchers for the 35 cultivated populations examined are given in Appendix 1, and vouchers for the 87 wild populations are noted in Appendix 2. Wild populations were found in roadside areas, often along very steep road cuts and other protected sites where plants were able to find refuge from the severe grazing pressure which has devastated much of the landscape of Turkey. Probably some of the "wild" populations represented escapes from cultivation, since domesticated alfalfa is very widespread in Turkey and was often observed to be close to plants growing outside of cultivation. The populations cited here were usually mass gatherings from available fruiting material, which in some cases represented many plants and in others very few. In several instances, very little if any distance separated the collections. As well as the material which served to provide cytological evaluation, several hundred herbarium collections were made during the trip and were considered in some of the interpretations made in the Discussion. The herbarium vouchers preserved in Ottawa contain a more extensive description of the collection sites than provided here, as well as other pertinent information. Two tables have been deposited in CISTI which give details of site location and numbers of plants evaluated cytologically.¹ Seeds are available for distribution for most of the populations for which USDA Inventory Numbers are given in the Appendixes.

Results

All 35 cultivated collections (Appendix 1) proved to be tetraploid ($2n = 4x = 32$). All were purple-flowered and had coiled pods, indicating little if any infiltration of *M. sativa* ssp. *falcata* germplasm.

Chromosome determinations made in wild plants were either diploid or tetraploid (Fig. 1). Tetraploid wild plants, assignable to subspecies *sativa* and subspecies \times *varia*, were very common in all areas traversed. "Pure" tetraploid subspecies *falcata* populations were rarely encountered (Appendix 2). Collection 17 produced what may be interpreted as tetraploid subspecies *falcata*, but growing adjacent to it was collection 16, an example of tetraploid subspecies \times *varia*. In many areas of Turkey highly polymorphous populations were encountered, with segregating flower colors indicative of recent hybridization between subspecies *falcata* and subspecies *sativa*. Moreover, the progeny raised in Beltsville of single plants collected in Turkey were occasionally also notably divergent from their parents, indicating that hybridization between rather divergent forms had occurred.

Diploid plants were discovered only in northeastern Turkey (Appendix 2, Fig. 2). These often grew in close proximity to tetraploids, and two of the mass collections proved to be mixtures of diploids and tetraploids. Several acquisitions of "pure" diploid subspecies *falcata* and subspecies *caerulea* were obtained. As in areas of tetraploid distribution, intermediate plants (assignable to subspecies \times *varia*) were found. At the common site of collections 107 (*falcata*) and E-79 (*caerulea*, *falcata*, and their possible hybrid), a hybrid zone was present, and chromosome complements of the parental and hybrid variants present were all diploid (Appendix 2).

¹Tables 2 and 3 for this paper are available, at a nominal charge, from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Ont. Canada K1A 0S2.

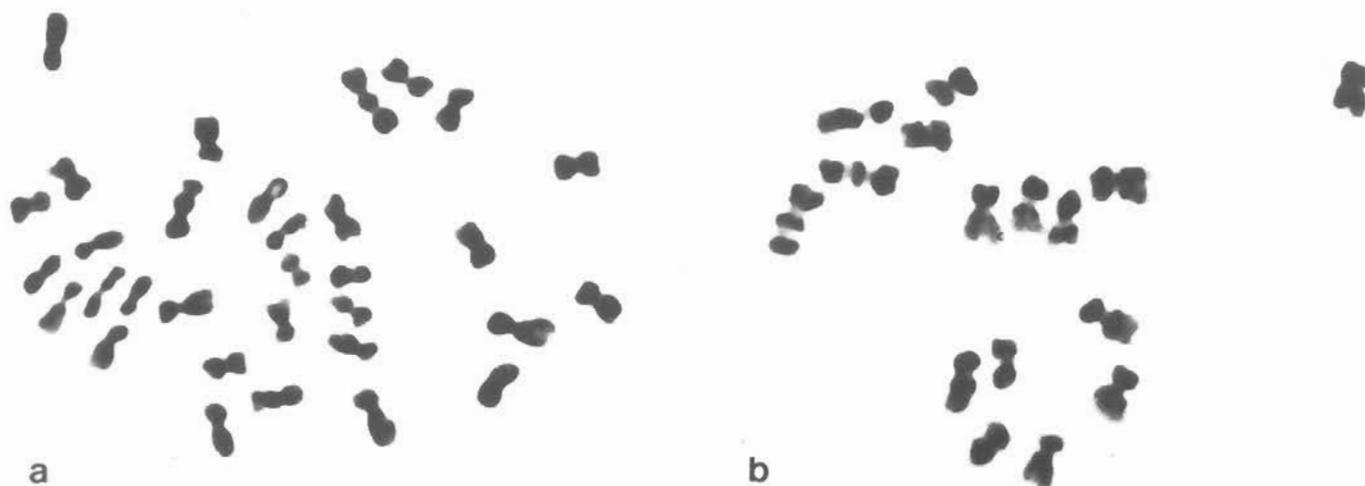


FIG. 1. Somatic chromosomes of (a) subspecies *sativa* (collection 80, $2n = 4x = 32$), and (b) subspecies *caerulea* (collection 105, $2n = 2x = 16$), $\times 2000$.

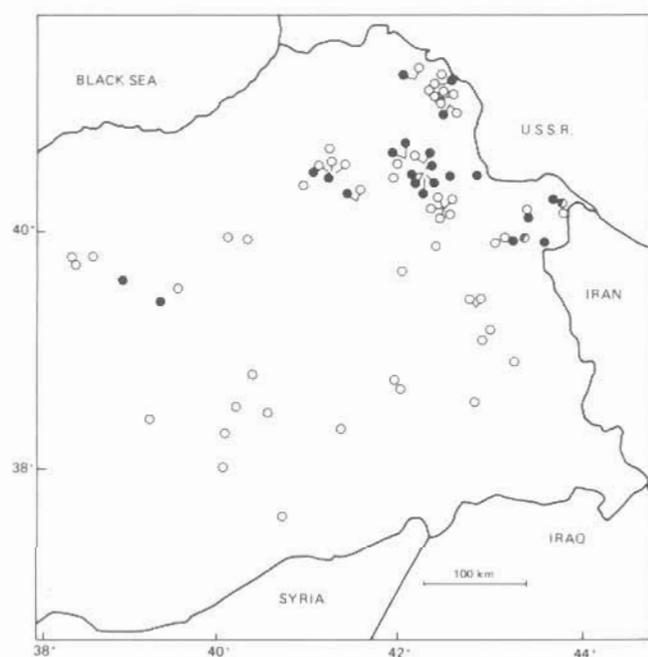


FIG. 2. Locations and ploidy level of wild collections of the *Medicago sativa-falcata* complex made in eastern Turkey; \circ , tetraploids ($2n = 4x = 32$); \bullet , diploids ($2n = 2x = 16$); \ominus , mixed collections. Where circles are displaced for clarity, exact location is indicated at end of the line extending from circle. All collections made west of area shown were tetraploid.

Discussion

Several of the observations noted here are in accord with previous records of the ploidy level variation in the *Medicago sativa* complex (as summarized in Lesins and Lesins 1979). Thus domesticated alfalfa is almost completely tetraploid (although it was interesting that this was so consistent even in areas of primitive agriculture). Wild tetraploid subspecies *sativa*, \times *varia*, and *falcata* are widespread in Eurasia, and at least the first two proved so in Turkey. Davis' (1970) account of subspecies *caerulea* indicates that the few specimens known to him were present in northeastern Turkey; indeed, we found diploid plants assignable to subspecies *caerulea* to be restricted

to the northeast. This is consistent with the view that northeastern Turkey represents the western most natural distribution area of diploid purple-flowered *M. sativa* (Ivanov 1977, 1980).

It is evident that in Turkey there is extensive gene exchange between variants at the tetraploid level in wild representatives of the *M. sativa* complex; although not as evident, a parallel situation apparently exists at the diploid level. A study of the distribution of some 32 diploid populations of subspecies *falcata*, as well as of diploid populations presumably of subspecies *caerulea* and some apparent diploid hybrids between the two (under the name *M. hemicycla* Grossheim) was carried out by Lesins and Lesins (1964). None of the material these authors employed originated from Turkey. Lesins and Lesins' study is in accord with ours in indicating that at least some spontaneous hybridization occurs at the diploid level.

The discovery of mixed diploid-tetraploid populations, and indeed the observation that in northeastern Turkey the two are rarely geographically isolated, raises the possibility of gene exchange between them. Stanford *et al.* (1972) reported that crosses between diploid and tetraploid alfalfa are successful only about 1% of the time in producing hybrids, the progeny substantially being tetraploids and sometimes triploids. If there is natural gene flow between the chromosome levels, it is likely to be much greater from the diploids into the tetraploids, because of the possibility of reduced pollen formation. Lesins and Lesins (1979, p. 97) indicated that chromosome reduction as well as increase can occur in polyembryonic seeds, and they postulated that both the increase and decrease in chromosome number may take place spontaneously in natural populations. Although the diploids and tetraploids of the *M. sativa* complex are often geographically and ecologically separated (Lesins and Lesins 1979), the bulk of the diploid populations that we collected in northeastern Turkey gave little evidence of natural geographical or habitat breeding barriers from each other or from the tetraploids. Indeed, the difficulties of consistently separating morphologically all populations of diploid subspecies *caerulea* from all populations of tetraploid subspecies *sativa* (Davis 1970; Lesins and Lesins 1979) could be a result of introgressive hybridization.

The task of morphological analysis of the collections remains. The extent of morphological discontinuity between and within the diploids and tetraploids may provide a means of

improving the presently uncertain classification of the *M. sativa* complex.

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- ALINOĞLU, N., H. MERTTURK, and A. T. OZMEN. 1972. Research on the prominent morphological and physiological characteristics of Kayseri alfalfa (*Medicago sativa* var. *Kayseri* N. A.). Grassland and Animal Husbandry Research Institute, Ankara, Turkey, Publ. No. 19.
- CHRISTIANSEN-WENIGER, F., and O. TARMAN. 1939. Anatolian lucerne. *Herb. Rev.* 7(2): 59–69.
- DAVIS, P. H. 1970. *Medicago* (perennials). In *Flora of Turkey and the East Aegean Islands*. Vol. 3. Edited by P. H. Davis. University Press, Edinburgh, pp. 488–490.
- FEDOROV, A. A. (Editor). 1969. Chromosome numbers of flowering plants. Academy of Sciences USSR, Moscow.
- GROSSHEIM, A. A. 1971. *Medicago*. In *Flora of the U.S.S.R.* Vol. 11. Edited by V. L. Komarov. Akademiya Nauk SSSR, Moscow and Leningrad, pp. 102–176. (Translated in 1971 by the Israel Program for Scientific Translations, Jerusalem.)
- GUNN, C. R., W. H. SKRDLA, and H. C. SPENCER. 1978. Classification of *Medicago sativa* L. using legume characters and flower colors. U.S. Dep. Agric. Agric. Res. Serv. Tech. Bull. No. 1574.
- IVANOV, A. I. 1977. History, origin, and evolution of the genus *Medicago*, subgenus *Falcago*. *Bull. Appl. Bot. Genet. Plant Breed.* 59(1): 3–40. (In Russian.)
- . 1980. Lucerne. Kolos, Moscow. (In Russian).
- LESINS, K. 1976. Alfalfa, lucerne. In *Evolution of crop plants*. Edited by N. W. Simmonds. Longman, London, pp. 165–168.
- LESINS, K., and I. LESINS. 1964. Diploid *Medicago falcata* L. *Can. J. Genet. Cytol.* 6: 152–163.
- . 1979. Genus *Medicago* (Leguminosae). A taxogenetic study. Dr. W. Junk by Publishers, The Hague.
- LUBENETS, P. A. 1972. Alfalfa—*Medicago* L. (A brief survey of the genus and the classification of the subgenus *Falcata* (Rehb.) Grossh.). *Bull. Appl. Bot. Genet. Sel. Forage Crops*, 47(3): 1–68.
- SINSKAYA, E. N. 1950. Perennial leguminous plants, part I: medic, sweetclover, fenugreek. In *Flora of cultivated plants of the U.S.S.R.* Vol. 13. Edited by E. N. Sinskaya, pp. 1–256. (Translated in 1961 by the Israel Program for Scientific Translations, Jerusalem.)
- SMALL, E. 1981. An alfalfa germ plasm expedition in Turkey. *Forage Notes*, 25(2): 56–66.
- . 1982. *Medicago* collecting in Turkey. *Plant Genet. Res. Newsl.* 49: 11–12.
- . 1983. Pollen ploidy-prediction in the *Medicago sativa* complex. *Pollen Spores*, 25. In press.
- SMALL, E., and B. S. BROOKES. 1984. Taxonomic circumscription and identification in the *Medicago sativa-falcata* (alfalfa) continuum. *Econ. Bot.* 38. In press.
- SNOW, R. 1963. Alcoholic hydrochloric acid — carmine as a stain for chromosomes in squash preparations. *Stain Technol.* 38(1): 9–13.
- STANFORD, E. H., W. M. CLEMENT, JR., and E. T. BINGHAM. 1972. Cytology and evolution of the *Medicago sativa-falcata* complex. In *Alfalfa science and technology*. Edited by C. H. Hanson. American Society of Agronomy, Madison, WI, pp. 87–101.
- TJIO, J. H., and A. LEVAN. 1950. The use of oxyquinoline in chromosome analysis. *Anal. Est. Exp. Aula Dei*, 2: 21–64.
- VASSILCZENKO, I. T. 1949. Lucerne. *Acta Institutii Botanici nom. V. L. Komarovii*, Ser. 1, Fasc. 8: 1–240. (In Russian.)

Appendix 1

This appendix is an abbreviation of Table 2, placed in the Depository of Unpublished Data, CISTI. This table gives details of altitude, latitude, longitude, numbers of plants evaluated, and whether evaluated meiotically or mitotically, and provides some information on nature of cultivar.

The following are the collection numbers ("E. Small *Medicago*—Turkey" numbers under which herbarium voucher specimens are deposited at Herbarium of Department of Agriculture at Ottawa) and United States Department of Agriculture Plant Inventory Numbers (in parentheses) of cultivated alfalfa accessions collected in Turkey. All accessions were found to be tetraploid ($2n = 4x = 32$).

2 (464730), 4 (464731), 7 (464733), 9–13 (464734–464738), 18 (464739), 19 (464730), 24 (464743), 36 (464748), 37 (464749), 40 (464751), 41 (464752), 42 (464753), 47 (464754), 48 (464755), 58 (464759), 60 (464761), 61 (464762), 62 (464763), 63 (464764), 66 (464765), 67 (464766), 71 (464769), 75 (464772), 102 (464781), 127 (464783), 130 (464785), 142 (464787), 148 (464791), 166 (4647940), 173 (464795), 178 (464796).

Appendix 2

This appendix is an abbreviation of Table 3, placed in the Depository of Unpublished Data, CISTI. This table gives details of altitude, latitude, longitude, numbers of plants evaluated, whether plants were collected in field or grown in greenhouse from seed, and whether evaluated meiotically or mitotically.

The following are the collection numbers ("E. Small *Medicago*—Turkey" numbers under which herbarium voucher specimens are deposited at Herbarium of Department of Agriculture at Ottawa), United States Department of Agriculture Plant Inventory Numbers, and ploidy level of wild alfalfa accessions collected in Turkey.

Medicago sativa subsp. *sativa* ($2n = 4x = 32$): 5 (464732), 21 (464741), 23 (464742), 27 (464744), 28 (464745), 32 (464746), 35 (464747), 38 (464750), 54 (464797), 59 (464760), 69 (464767), 70 (464768), 72 (464770), 74 (464771), 76 (464773), 77 (464774), 78 (464775), 79 (464776), 80 (464777), 82 (464778), 84 (464779), 90 (464710), 91 (464711), 101 (464780), 126 (464782), 128 (464784), 133 (464786), 144 (464788), 147 (464790), 152 (464792), 164 (464793), 181 (464797), 182 (464798), 183 (464799), E-57, E-73, E-86, E-122, E-133, (464790).

Medicago sativa subsp. *falcata* ($2n = 4x = 32$): 17 (464725), 171a.

Medicago sativa subsp. *falcata* ($2n = 2x = 16$): 107, 109 (464726), 110 (464727), 111 (464728), 112 (464729), E-79c, E-81, E-85, E-90.

Medicago sativa subsp. \times *varia* ($2n = 4x = 32$): 16 (464800), 20 (464801), 33 (464802), 113 (464803), 115 (464804), 116 (464805), 119 (464806), 120 (464802), 125 (464808), 161, 165 (464809), 171c, 171d, 172 (464810), 175 (464811), 176 (464812), 179 (464813), 187 (464814), E-80, E-83, E-88, E-89, E-128, E-131.

Medicago sativa subsp. *caerulea* ($2n = 2x = 16$): 92 (464712), 94 (464714), 97, 99 (464715), 103 (464717), 104 (464718), 105 (464719), 106 (464720), 129 (464721), 143 (464722), 145 (464789), 159 (464723), 160 (464724), E-79b, E-79c.

Medicago sativa subsp. *caerulea* \times subsp. *falcata* ($2n = 2x = 16$): E-79a, E-79d.

Mixed populations (*Medicago sativa* subsp. *caerulea*) ($2n = 2x = 16$) + subsp. *sativa* ($2n = 4x = 32$): 93 (464713), 100 (464716).