

Cytological studies of callus, *in vivo* and *in vitro* cells of a new variety of *Onobrychis viciifolia*

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Abstract Summary: The present research focused on the cytological studies of Embryogenic (E) and Non-Embryogenic (NE) callus as well as root meristem cells of *in vivo* and *in vitro* grown plants as the main tissues of *Onobrychis viciifolia* species.

Introduction: Description of the regenerative behavior of somatic cells is an important prerequisite to reveal the ways in which cell proliferation is regulated, and how the non-organized cells, i.e., callus, can be stimulated to embryonic organization. The different behavior of embryogenic and non-embryogenic cells can be detected by noticeable differences within their cells. Cytological analyses are usually performed to assess the mitotic process in experimental varieties and hybrids. Despite the existence of some few cytological studies of *Onobrychis viciifolia* and little work rarely focused on detailed karyological criteria (Mesicek and Sojak 1992), no research has been conducted on callus cellular behavior.

Materials and Methods

Plant Materials: Seeds of *Onobrychis viciifolia* existing from natural- agricultural resources of Iran were selected where was situated at 34° 64' North and 50° 78' E longitude with an elevation of 930 meters above the mean sea level.

Growth Culture: For *in vitro* growth condition, 100% of seeds germinated in the Murashige and Skoog medium (MS) supplemented with 3% (w/v) sucrose and 0.75% (w/v) agar after 1-2 weeks. The seeds and containers were not sterilized in *in vivo* growth culture and the samples were germinated on soiled vase in greenhouse.

Callus Production: After 3-4 weeks of incubation of seed cultures in MS media, stem explants of sainfoin were cut into small pieces (2-3 mm). To induce the callus, the explants were inoculated in MS medium fortified with different concentrations of BAP, NAA and BAP, IBA (Mohajer et al. 2012). To ensure that the NE callus had truly lost regeneration capacity and did not contain the E cells, double staining method (Gupta et al. 1987) was employed.

Cytometric Parameters: Permanent slides of *in vitro*, *in vivo* and callus tissues were analyzed for cellular behavior, such as Mitotic Index (MI), chromosome counts, mean nuclear and cell areas, and their ratios (Evans and Van't Hof, 1974).

Results: Either more than 50% of the cells were in G1 phase of the cell cycle or no significant difference between cells of the *in vitro* and *in vivo* growth was recognized (Table 1). Although the ratio of mean nuclear of cell area was larger in *in vitro* than intact plants, mean nuclear and cell areas of *in vivo* were approximately 2.5 and 3 times more than *in vitro* (Table 2). A different percentage of the mitotic irregularities observed in callus and also in both cultures included the occurrence of varied degree of laggards and bridge, binucleate cells, asynchronous nuclei, cytomixis and micronucleus (Table 3 and Fig. 1). The karyotype formulae, parameters and also idiogram of the studied species are illustrated in Figs. (2a, b).

Discussion: The significance of this study is unlike previous studies which introduce a diploid variety of *O. viciifolia* ($2n=2x=16$) rather than reported tetraploid varieties ($2n=4x=28$) by Ranjbar et al. (2010) for genetic diversity of this species. Goldblatt (1981) has hypothesized that chromosome number of $x = 7$ has been indeed derived from $x = 8$ which is hereditary in the genus. Hesamzadeh and Ziaie (2010) found the basic chromosome numbers of $x=7$ and $x=8$ for diploid genus and only $x=7$ was observed in tetraploid populations. In following, Ghanavati et al. (2012) observed $x=8$ only in the

tetraploid genus of the *Onobrychis*. Therefore, studied variety probably originates from a wild *Onobrychis* genus which has been mutated and finally adapted to the environmental stress. Since distribution of the most diploids due to the temperature is southwestern Asia (Ranjbar et al. 2010), variation in *viciifolia* is attributed to this region especially Iran.

Table 1. The percentage of the nuclei in interphase and polyploidy

Cell line	Cell cycle phase (%)			Polyploidy (%)
	G1	S	G2	
<i>In vitro</i>	55.31±0.32	34.04±0.15	10.63±0.11	-
<i>In vivo</i>	59.25±0.24	33.31±0.27	7.40±0.06	-
E* callus	9.67±0.17	41.93±0.25	19.35±0.14	29.03±0.15
Non-E.callus	32.25±0.22	29.03±0.13	6.45±0.09	32.25±0.24

Table 2. The mean cell (C) and nuclear (N) areas of different growth conditions

Growth condition	Cell (µm ²)	Nuclear (µm ²)	N/C
<i>In vitro</i>	70683.72bc ± 26	5984.25bc ± 6	0.089
<i>In vivo</i>	232080.28a ± 32	12051.26a ± 18	0.062
E* callus	74563.84bc ± 12	12524.21a ± 24	0.186
Non-E.callus	60989.84c ± 29	8352.97b ± 11	0.137

Table 3. Mitotic aberrations found in different growth conditions

Growth conditions	Cytomixis (%)	Bridge/Laggard (%)	Micronucleus (%)	Asynchronous nucleus (%)	Binucleated cells (%)
<i>In vitro</i>	-	0.53	0.42	0.49	0.97
<i>In vivo</i>	1.21	1.28	0.09	0.05	2.47
E* callus	2.65	0.88	0.19	0.08	0.09
Non-E.callus	3.41	3.47	1.15	0.08	1.18

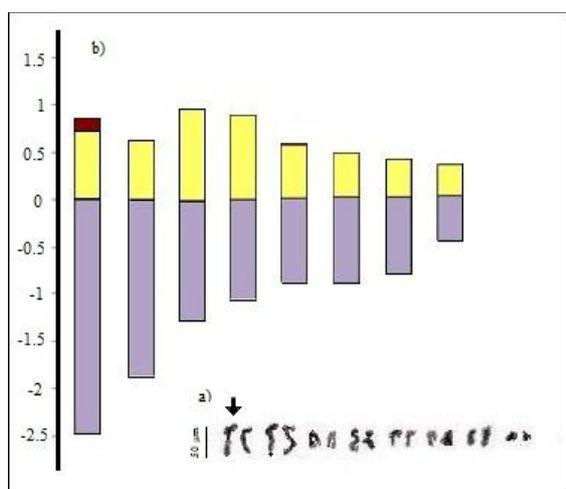


Fig. 1. a) Karyotypes and b) Ideogram
Arrows indicate the satellite. Bar = 50µm

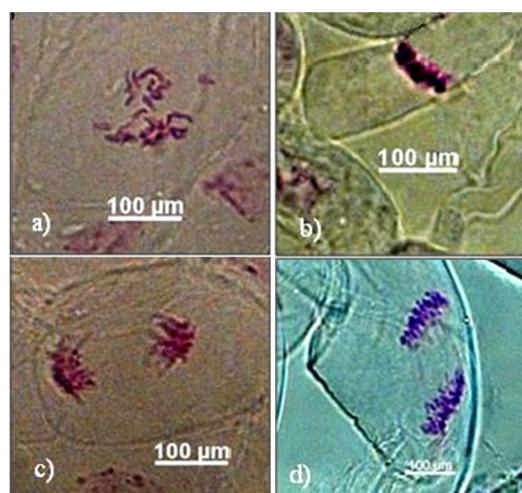


Fig. 2. Representative normal and abnormal mitotic cells
a. Prophase; b. Metaphase; c. Anaphase; d. Telophase

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