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Izmir subterranean clover (*Trifolium subterraneum* L. var. *subterraneum*)

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Abstract. Izmir is a hardseeded, early flowering, subterranean clover of var. *subterraneum* (Katz. et Morley) Zohary and Heller collected from Turkey and developed by the collaborating organisations of the National Annual Pasture Legume Improvement Program. It is a more hardseeded replacement for Nungarin and best suited to well-drained, moderately acidic soils in areas with a growing season of less than 4.5 months. Izmir seed production and regeneration densities in 3-year pasture phases were similar to Nungarin in 21 trials across southern Australia, but markedly greater in years following a crop or no seed set. Over all measurements, Izmir produced 10% more winter herbage and 7% more spring herbage than Nungarin. Its greater hardseededness and good seed production, makes it better suited to cropping rotations than Nungarin. Softening of Izmir hard seeds occurs later in the summer–autumn period than Nungarin, giving it slightly greater protection from seed losses following false breaks to the season. Izmir is recommended for sowing in Western Australia, New South Wales, Victoria, South Australia and Queensland. Izmir has been granted Plant Breeders Rights in Australia.

Origin

Izmir is derived from a wild population of subterranean clover collected on 29 June 1987 by C. M. Francis (Department of Agriculture and Food Western Australia) on the eastern outskirts of Emiralem village in Izmir Province, Turkey. The collection site, given the code CIZ008, was located in a heavily grazed area among olive trees on a roadside terrace. The soil was a well-drained, friable, stony, grey-brown sandy loam of basaltic origin with a pH in water of 6.5–7.0. The site was also densely populated by other annual legumes, notably *Trifolium batmanicum*, *T. pauciflorum*, *T. glomeratum*, *T. spumosum*, *T. scutatum*, *Medicago polymorpha* and *M. praecox*. Estimated altitude was 110 m above sea level with a mean annual rainfall of 700 mm.

Initially known as CIZ008Sub-G, Izmir was 1 of 10 distinct subterranean clover genotypes identified from site CIZ008 in 1987 at South Perth, Western Australia by K. D. Foster (Department of Agriculture and Food Western Australia). The distinctness of these genotypes was confirmed at South Perth in 1988 in 1-m rows sown to 0.5 g of seed. In 1990, Izmir was screened at Shenton Park and Wongan Hills, Western Australia for early flowering, low formononetin content (less than 0.2% of dry matter), using the procedures of Francis and Millington (1965), and hardseededness after 4 months in a fluctuating 60/15°C temperature cabinet, using the procedure of Quinlivan (1961).

In 1991, Izmir was selected as 1 of 104 early maturing breeding lines for Stage I field evaluation in Western Australia, South Australia, Victoria, and Queensland. Initial evaluation in New South Wales commenced in 1993. Izmir was the best performing line in Western Australia and was selected as 1 of 16 breeding lines for national Stage II trials in 1995.

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Izmir was evaluated under the code name CIZ008Sub-G in Stage I trials, and as SE008 in Stage II trials. Testing was conducted as part of the National Annual Pasture Legume Improvement Program (NAPLIP). The following collaborators of NAPLIP conducted field evaluation and final selection of Izmir: P. G. H. Nichols and P. Si (Department of Agriculture and Food Western Australia), G. A. Sandral and B. S. Dear (New South Wales Department of Primary Industries), C. T. de Koning and A. D. Craig (South Australian Research and Development Institute), D. L. Lloyd (Department of Primary Industries and Fisheries, Queensland) and P. M. Evans (Department of Primary Industries Victoria). M. J. Barbetti, D. J. Gillespie and M. P. You (Department of Agriculture and Food Western Australia) conducted screening for disease resistance. D. J. Gillespie conducted screening for red-legged earth mite [*Halotydeus destructor* (Tucker)] resistance. P. G. H. Nichols and P. F. Smith (Centre for Legumes in Mediterranean Agriculture) conducted hardseed screening. The University of Western Australia conducted isoflavone analyses. Further details of trial sites and the field evaluation process are given in Sandral *et al*. (1998).

Izmir was selected for release as a new cultivar in July 2001 by P. G. H. Nichols and B. J. Nutt (Department of Agriculture and Food Western Australia), G. A. Sandral, B. S. Dear, C. T. de Koning, A. D. Craig, P. M. Evans and D. L. Lloyd. It is recommended for registration by the collaborating organisations of NAPLIP. Selection criteria included early flowering, high hardseededness, low formononetin content, greater herbage production and persistence than cultivar Nungarin and high seed production. Breeders' seed is based on 26 plants selected for uniformity at Shenton Park in 2001. Izmir has been granted Plant Breeders Rights in Australia and is described in Nichols (2006). The Department of Agriculture and Food Western Australian will maintain breeders' seed.

Izmir is named after its collection site in Izmir province, Turkey.

Morphological description

Izmir belongs to the taxon *Trifolium subterraneum* var. *subterraneum* (Katz. et Morley) Zohary and Heller. It has a relatively prostrate growth habit, giving it good grazing tolerance. Izmir has a leaf mark of C_3 (A_1), using the classification of Nichols *et al*. (1996), consisting of a central light green C_3 crescent extending almost to the margins, with faint white A_1 arms that fade in spring, located beneath the crescent and extending to the margins. Under cold and other growth-limiting conditions, leaflets produce a purplish brown anthocyanin flush proximal to the crescent along the midrib and often extending to the margins. Anthocyanin flecking is absent. Indentation of distal margins is weak. Stipule pigmentation under closed canopies is weak–intermediate. Calyx tubes have a reddish-purple pigmentation extending three quarters of the length from the distal end. Stems and peduncles are strongly pubescent, leaf upper surfaces are weakly–moderately pubescent, whereas petioles have few hairs. Burr burial is moderately strong. Seeds are black, with about 120 000 seeds per kg when grown under ideal conditions. Further descriptions and photographs of distinguishing features are given in Nichols (2006).

Agronomic characters

Flowering time

Izmir is a very early flowering cultivar. In Perth, it flowers about 78 days after sowing in early May, which is about 1–2 days later than Nungarin, about 20 days earlier than Dalkeith and 25 days earlier than Urana (Nichols 2004). Its early maturity makes Izmir well suited to low rainfall, short-growing season environments. Fresh leaves in spaced plants of Izmir contain a low level (about 0.1% of dry matter) of the oestrogenic isoflavone, formononetin, whereas levels of genistein and biochanin-A are 0.9 and 0.4% of dry matter, respectively. The low level of formononetin, indicates a low potential for Izmir to cause sheep infertility problems.

Hardseededness

Both laboratory and field measurements indicate that Izmir is more hard-seeded than other subterranean clover cultivars. In laboratory tests averaged over six seasons, Izmir seeds derived from 1-m single rows grown at Shenton Park, had 67% hardseed after 16 weeks in a 15°/60°C cabinet, using the standard procedures of Quinlivan (1961), whereas Urana, Dalkeith, Nungarin, Geraldton and Daliak had 63, 56, 55, 42 and 38% hardseed, respectively. In a field test at Shenton Park, 39% of Izmir seeds grown at Nungarin and Naraling, Western Australia were still hard after 5 months of softening, compared with 35% for cultivar Nungarin, 29% for Urana, 27% for Geraldton, 25% for Dalkeith and 23% for Dwalganup (Norman *et al*. 2006). After 30 months in the field, a mean of 5% of Izmir seeds were still hard, compared with 3.5% of Nungarin, 2% of Geraldton, 1.5% of Dalkeith and Urana, and 1% of Dwalganup. This level of hardseededness should enable Izmir to persist more reliably than other early flowering cultivars in low rainfall pasture–crop rotations or in environments with unreliable seed-setting conditions.

The timing of seed softening in the field over the summer–autumn period is slightly delayed in Izmir compared with other early flowering cultivars, giving it greater protection from seed losses following false breaks to the season. The majority of Izmir hardseeds soften about 2 weeks later than Nungarin, Geraldton and Urana and at a similar time to Dalkeith (Norman *et al*. 2006). Most seed softening of Izmir is completed by early April.

Disease and pest resistance

Izmir is susceptible to Race 1 of clover scorch disease caused by *Kabatiella caulivora* (Kirchn.) Karak. In a Race 1 field screening trial at Mt Barker, Western Australia, Izmir had a disease severity rating of 6.0 compared with scores of 7.5 for Dalkeith, 7.0 for Nungarin, Urana and Seaton Park and 2.5 for Daliak (increasing disease severity scale of 0–10). Preliminary field and glasshouse data suggests Izmir is at least moderately susceptible to Race 2. Izmir is highly susceptible to cercospora leafspot (*Cercospora zebrina* Pass.). Inoculated field plots of Izmir had similar cercospora leafspot incidence and leaf collapse ratings to Nungarin, Dalkeith and Urana (Barbetti and Nichols 2005). Izmir is also moderately susceptible to leaf rust (*Uromyces trifolii-repentis* Liro). Izmir had a damage rating of 6.5 in a natural outbreak of the disease in field plots

Table 1. Mean field performance data of Izmir subterranean clover (as a percentage of Nungarin) in 21 field trials across southern Australia
Not all traits were measured at each site in every year

Cultivar	Herbage production		Seed bank		Seedling regeneration density	
	Winter	Spring	Continuous pasture	Year after no seed set[A]	Continuous pasture	Year after no seed set[A]
Izmir	110	107	103	175	98	134
Nungarin	100	100	100	100	100	100

[A] Two sites cropped and two sites sprayed out before seed set.

at Shenton Park, compared with 6.5 for Dalkeith, 5.1 for Urana, 4.6 for Nungarin and 3.2 for Geraldton. The reaction of Izmir to root rotting pathogens has not yet been determined. Diseases are rarely encountered in the low rainfall, short growing season target area for Izmir and are unlikely to present problems to graziers. However, seed growers in high rainfall, disease-prone areas may need to control diseases under ideal epidemic conditions.

Izmir is similar in susceptibility to red-legged earth mite, *H. destructor*, to other cultivars, particularly at the cotyledon stage. Growth room tests on 2-week-old seedlings gave mean cotyledon damage ratings of 5.2 for Izmir, 4.1 for Urana, 4.9 for Nungarin, 5.8 for Geraldton and 6.1 for Dalkeith (on an increasing damage severity rating of 0–10).

Field performance

Field performance of Izmir was measured in 21 trials at low rainfall (<375 mm) sites across southern Australia (Sandral *et al*. 1998). Overall field performance was superior to Nungarin, a common treatment at each site, and is shown in Table 1. Izmir produced 10% more winter herbage and 7% more spring herbage than Nungarin. Izmir also produced 4% more winter herbage overall than the later flowering cultivars Dalkeith and Urana, but spring production of Izmir was only 98% of Dalkeith and 87% of Urana.

Mean seed banks of Izmir in the first 2 years of a continuous pasture phase were slightly greater (3%) than Nungarin (Table 1). At Wongan Hills, Western Australia, where seed banks were measured after 3 seasons, Izmir had 54% more seed than Nungarin, 92% more than Dalkeith and more than 4 times that of Urana. The hardseeded advantage of Izmir was apparent following seasons where trials were either cropped (two sites) or sprayed out to prevent seed set (two sites). Where this occurred, overall mean seed reserves of Izmir were 75% higher than both Nungarin and Dalkeith and nearly 3-times greater than Urana.

Overall seedling regeneration densities of Izmir were 98% of Nungarin in continuous pastures (Table 1). This was largely attributable to its lower densities (96%) in the year following establishment, presumably as a result of its higher hardseededness. Year 3 regeneration densities of Izmir were slightly higher than Nungarin (101%). The advantage of the higher hardseededness of Izmir was demonstrated following seasons in the 4 trials where seed set was prevented. In these instances, overall mean seedling regeneration density of Izmir was 34% higher than Nungarin (Table 1), 12% higher than Dalkeith and more than twice that of Urana.

Potential use

Izmir is a more hardseeded replacement for Nungarin and is suited to well-drained, moderately acidic (pH_{Ca} 4.5–6.5) soils in low rainfall, short growing season areas, where other cultivars reach maturity too late for reliable seed setting and persistence. Typical areas have a growing season length of less than 4.5 months and less than 375 mm annual rainfall, although this varies across districts. The higher hardseededness of Izmir, coupled with its good seed production, should enable it to persist better than other subterranean clovers in cropping rotations. However, excessive cropping and inadequate management during the pasture phase will reduce its seed bank to unsustainable levels, albeit at a slower rate than other cultivars. Izmir is likely to be used in mixtures with Dalkeith, particularly in wetter areas of the target zone.

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Seed licenced to: Premier Seeds, PO Box 40, Forbes, NSW 2871, Australia and Ballard Seeds, Box 7, Tincurrin, WA 6361, Australia.

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