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Spontaneous outcrossing in tomato depends on cultivar and environment and varies between individual flowers

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Abstract

Knowledge about the degree of spontaneous outcrossing of diverse genotypes is essential for breeding programmes, maintenance breeding, and seed production. For tomato (*Lycopersicon esculentum* Mill.), very limited scientific evidence for genotypic differences is available and evidence from Europe is scarce. To close this knowledge gap, six cultivars were investigated in three Central European locations as part of the Organic Outdoor Tomato Project. To determine outcrossing rates, the monogenetic "cut-leaf" trait, which is dominant over the "potato-leaf" trait, was used as morphological marker. The observed range of outcrossing was 0.0%–5.2%. Outcrossing was significantly influenced by cultivar and environment. The outcrossing rate of individual flowers varied within cultivars ranging from 0% to 37%. The potential of newly opened flowers to accept foreign pollen varied largely with the cultivar. Genotypic differences could partly be linked to flower morphology traits. The potential for recombination between tomato genotypes is generally very low but can be a source for new variation in *on-farm* management.

KEYWORDS

breeding, cross-pollination, *Lycopersicon esculentum*, outcrossing, pollinators, seed production, *Solanum lycopersicum*

1 | INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is the most important vegetable globally as well as in Central Europe (FAO 2017). Tomatoes are grown on every continent and in an extremely wide range of environments. Cropping systems range from organic low-input outdoor production to hydroponic greenhouse systems with complete ambient control.

Tomato fruits appeal to our senses with a rich array of flavours, shapes, sizes, colours and colour patterns. This diversity has led to a large number of cultivars available from seed companies, NGOs, seed savers and genebanks. In collections, many genotypes are often grown in close proximity to each other for the purpose of seed production. Knowledge about the degree of outcrossing is relevant in order to decide when cross-pollination needs to be avoided by spatial or technical isolation. On the other hand, spontaneous outcrossing is often the source of variation within traditional cultivars of tomato (Cortés-Olmos, Valcárcel, Roselló, Díez, & Cebolla-Cornejo, 2015).

Tomato is generally treated as an autogamous species (Kaul, 1991; Lindhout, 2005; Veeraragavathatham, Auxcilia, Shoba, & Amarnath, 2005). According to Lindhout (2005), cross-pollination by wind and insects may occur in tomato. Flowers are generally not very attractive to insects (Gladis, Hammer, Dathe, & Pellmann, 1996; Quiros & Marcias, 1978). Honey bees visit tomato flowers in greenhouses when they have no choice, but tomato flowers outdoors are rarely visited (Free, 1993). Deprá, Girondi Delaqua, Freitas, and Gaglianone (2014) observed that bees visited tomato flowers more frequently than other insects did. Cross-pollination is apparently largely resulting from buzz pollination by native pollinators (De Melo e Silva Neto et al., 2013). In Central Europe, both bumble bees and solitary bees are the main pollinators (Teppner, 2005).

Outcrossing rates can vary strongly between regions of the world and to a lesser extent between locations on a smaller scale. Generally, higher outcrossing rates were observed in hot climates (Richardson & Alvarez, 1957; Rick, 1958; Singh, Singh, & Kanwar, 2012; Veeraragavathatham et al., 2005). The highest values were observed in the region of origin of tomatoes, that is north-western South America. Thus, in Southern Peru Rick (1958) observed outcrossing rates of 14.8% in Tacna Valley and 25.7% in Calana. Rick (1950) used a male-sterile genotype to assess relative cross-pollination and found that the average number of seeds per flower harvested on male-sterile plants in two locations in Peru was 14.2 and 14.8 compared to only 0.3 to 7.0 for 16 environments (years × locations) in California. Also, Richardson and Alvarez (1957) observed 3.3% to 7.0% outcrossing at three locations in Mexico and related the observed differences to the abundance of pollinators. At least in parts of the research area "wild tomatoes" were "rather common." Quiros and Marcias (1978) observed only 1.2% outcrossing in a "less tropical" region of Mexico.

Very limited scientific evidence is available for European conditions: no outcrossing (0%) was observed in Central and Southern Italy (Ilardi & Barba, 2002). An effect of the cultivar on outcrossing rates has been observed (Lesley, 1924), but evidence is scarce.

The study presented here is part of the Organic Outdoor Tomato Project. The project started in 2003 at the University of Göttingen, Germany, as a participatory organic plant breeding programme that involves the entire value chain (Horneburg, 2010). It is based on the free exchange of knowledge and tomato genotypes and improves organic plant breeding methods (Horneburg & Becker, 2011). Crosses are produced after a careful choice of parent genotypes. Additionally, genotypes deriving from spontaneous outcrossing were successfully included in the breeding programme. This indicates that recombination by spontaneous outcrossing and subsequent selection can contribute to develop future genetic resources and to select improved genotypes. Frequently, cultivars bred in the network are released (e.g., Kotschi, Rehberg, & Horneburg, 2017).

Knowledge about the degree of spontaneous outcrossing of diverse genotypes is essential for breeding programmes, maintenance breeding, and seed production. For tomato (*Lycopersicon esculentum* Mill.), very limited scientific evidence for genotypic differences is available and evidence from Europe is scarce. This study aimed to close this knowledge gap. Our research is based on about 24,000 pollination events. To make that possible we had to use a trait that can be scored at an early developmental stage to distinguish between seeds derived from self-pollination and seeds derived from outcrossing. Outcrossing was calculated in cultivars of the potato-leaf type which were grown surrounded by cultivars of the cut-leaf type. The cut-leaf trait, also named tomato leaf, is typical for most tomato cultivars and monogenetic dominant over potato-leaf (Lesley, 1924). Thus, all cut-leaf seedlings derived from a potato-leaf mother plant are the result of outcrossing.

2 | MATERIALS AND METHODS

Outdoor tomato trials were established in three organic market gardens in Central and Northern Germany: Saatgutgärtnerei Schönhagen (51°20′25.4″N, 10°01′12.6″E; certified by Demeter) deep clayey loam, altitude 310 m above sea level, average annual precipitation 550 mm, average annual temperature 7.5°C; Ellingerode (51°19′49.9″N, 9°49′14.1″E; market garden Wember, Bioland) shallow loam, 200 m, 650 mm, 8.5°C; Rhauderfehn (53°10′37.8″N, 7°32′14.5″E; market garden Lühring, Naturland) sand close to ground water, 2 m, 780 mm, 7.5°C. Six cultivars of the potato-leaf type were used to assess spontaneous outcrossing (Table 1).

The experiments were performed at all three locations in 2005 and at Schönhagen in 2006. To compare years, climate data for Schönhagen are given in Table 2. The plants were sown on 31 March 2005 and 4 April 2006, respectively, in a greenhouse according to organic standards in multipot trays QP 96 (Hermann Meyer KG, Germany). They were transferred to 1-l pots on 25 April 2005 and 19 April 2006. After the first two true leaves were developed, the plants were planted in the field trials on 25 May 2005 and 19 May 2006. Plants were spaced 1 × 1 m and pruned to the main shoot supported by a stick. From each cultivar, two plants were grown side by side, repeated twice in 2005 and three times in 2006. Next to each potato-leaf plant was one plant of the same cultivar plus seven cut-leaf plants. Thus, outcrossing was underestimated by 1/8, and the results were corrected accordingly. Pollination by another potato-leaf plant further away was not considered in this study because cross-pollination mainly occurs between adjacent plants (Veeraragavathatham et al., 2005).

Starting in July, one fruit per plant was harvested every 14 days until the beginning of October when frost terminated the trials. Late blight reduced the yield; at Ellingerode in 2005 and in Schönhagen 2006, some fruits were lost. They were replaced by fruits from other plants of the same cultivar in some cases. At Rhauderfehn in 2005, no fruits could be harvested from 'Domaca Pfarrgarten'.

At Schönhagen, one newly opened flower per plant was hand-pollinated on 29 June and 13 July 2005 to determine whether pollination had already occurred at this stage. A tuning fork was used to gather pollen from at least two well-developed flowers of different plants of a cut-leaf cultivar.

After harvest, seeds were cleaned and dried. All seeds were used to produce seedlings in the greenhouse. At the first true leaf stage, the leaf type, potato- or cut-leaf, was determined per offspring of single fruits. Outcrossing was calculated as the corrected percentage of potato-leaf seedlings. Analysis of variance and least significant

TABLE 1 Six potato-leaf tomato cultivars used to assess spontaneous outcrossing

Cultivar	Fruit weight (g)	Description of fruits	Seed source
Galina Siberian Cherry	17	Round, yellow	Privates SamenArchiv Gerhard Bohl, Germany
Quedlinburger Frühe Liebe	32	Round, red	Genebank of the IPK Gatersleben, Germany
Rosa Roma	46	Oval, pinkish red	Seed saver Jürgen Koch, Germany
Matina	50	Round, red	Dreschflegel, Germany
Ostravske Rane	51	Round, red	Genebank of the IPK Gatersleben, Germany
Domaca Pfarrgarten	247	Oblate, pinkish red, beefsteak	Arche Noah, Austria

TABLE 2 Climatic conditions Schönhagen 2005 and 2006

Mean per months		May	June	July	August
Temperature (°C)	2005	12.2	15.1	17.9	15.9
	2006	12.9	16.3	21.1	15.6
Precipitation (mm)	2005	82.5	49.5	70.1	63.0
	2006	64.6	56.6	71.6	77.3
Sunshine (h)	2005	196	239	203	158
	2006	210	256	333	122

Data according to DWD (2018), Wetterstation Göttingen, 51°50′03.3″N, 9°95′05.7″E.

difference of genotypic effects were calculated with PLABSTAT 3Awin (Utz, 2005). The effect of years and of individual fruits was analysed with the chi-squared test.

3 | RESULTS

3.1 | The effect of locations and cultivars

The assessment of outcrossing of six cultivars at three locations was based on >14,000 pollination events (Table 3). The mean outcrossing rate per location varied between 0.15% at Schönhagen and 0.45% at Rhauderfehn. Due to the small number of seedlings derived from outcrossing, differences between locations were not significant at $p = 0.1$. Due to climatic conditions and high disease pressure, the results for Rhauderfehn are based on a much smaller number of pollination events than for the other two locations. Cultivars differed significantly at $p = 0.01$ with a least significant difference of 0.55 ($p = 0.05$). The mean outcrossing rate per cultivar ranged from 0% in 'Galina Siberian Cherry' and 'Quedlinburger Frühe Liebe' to 1.39% in 'Domaca Pfarrgarten' (Table 3). The highest number of seedlings could be analysed from Galina Siberian Cherry (3,183) the lowest number from 'Domaca Pfarrgarten' (1,201).

3.2 | The effect of the year

The effect of the year was investigated at Schönhagen. The year had a highly significant effect on the outcrossing (Table 4) based on >10,000 pollination events. The outcrossing rate reached 2.9% in 2006 but only 0.03% in 2005. For each of the three cultivars investigated, a higher outcrossing rate was observed in 2006. The effect was not significant for 'Ostravske Rane'. In 'Rosa Roma' and 'Matina', no outcrossing had been observed in 2005.

3.3 | The effect of individual flowers within cultivars

The effect of single flowers was analysed in the environment with the highest outcrossing rate, that is Schönhagen 2006. In all three cultivars, individual flowers with an outcrossing rate exceeding the average values by more than 20% were observed (Table 5). The

TABLE 3 Outcrossing of six tomato cultivars at three locations in Germany, 2005

	Schönhagen				Ellingerode				Rhauderfehn				All locations			
	Fruits	Potato-leaf seedlings	Cut-leaf seedlings	Outcrossing (%) mean of cultivars	Fruits	Potato-leaf seedlings	Cut-leaf seedlings	Outcrossing (%) mean of cultivars	Fruits	Potato-leaf seedlings	Cut-leaf seedlings	Outcrossing (%) mean of cultivars	Fruits	Potato-leaf seedlings	Cut-leaf seedlings	Outcrossing (%) mean of cultivars
Location				0.15				0.37				0.45				0.32
Cultivar																
Galina Siberian Cherry	23	697	0	0.00	23	2,408	0	0.00	7	78	0	0.00	53	3,183	0	0.00
Quedlinburger Frühe Liebe	19	992	0	0.00	23	1,134	0	0.00	5	111	0	0.00	47	2,237	0	0.00
Rosa Roma	17	993	0	0.00	21	1,475	0	0.00	17	174	1	0.65	55	2,642	1	0.22
Matina	19	975	0	0.00	17	860	2	0.26	14	227	1	0.50	50	2,062	3	0.25
Ostravske Rane	20	1,058	1	0.11	23	1,723	0	0.00	8	130	0	0.00	51	2,911	1	0.03
Domaca Pfarrgarten	15	596	4	0.77	10	591	10	1.90	0[a]	0[a]	0[a]	1.52	25	1,187	14	1.39

[a]No value was obtained at Rhauderfehn; a missing value for outcrossing was estimated by PLABSTAT 3Awin.

TABLE 4 Outcrossing of three tomato cultivars 2005 and *2006* (*in italics*) at Schönhagen, Germany

Cultivar	Fruits		Potato-leaf seedlings		Cut-leaf seedlings		Outcrossing (%)		Chi-squared test [a]
	2005	2006	2005	2006	2005	2006	2005	2006	
Rosa Roma	17	*32*	993	*2,061*	0	*86*	0.00	*5.23*	40.9***
Matina	19	*36*	975	*2,510*	0	*56*	0.00	*2.85*	21.6***
Ostravske Rane	20	*34*	1,058	*2,285*	1	*11*	0.11	*0.63*	n.s.
Total	56	102	3,026	6,856	1	153	0.03	2.90	64.7***

[a]Test of years against average outcrossing per cultivar/total.
***Significant at p = .0001; n.s., not significant.

absolute number of seedlings deriving from outcrossing varied from 0 to 22.

3.4 | The potential to accept foreign pollen

The potential to accept foreign pollen was investigated at Schönhagen 2005 (Table 6) by hand-pollinating newly opened flowers with pollen from cut-leaf plants. In three cultivars, the resulting outcrossing rate was only 2.1% or less. In 'Matina', it was 24.6% and in 'Ostravske Rane' and 'Domaca Pfarrgarten', about half to three quarters of the seedlings resulted from outcrossing.

TABLE 5 Outcrossing of individual flowers of three cultivars Schönhagen, Germany, 2006

Cultivar	Potato-leaf seedlings	Cut-leaf seedlings	Outcrossing (%)	Chi-squared [a]
Rosa Roma	40	19	36.8	100.7***
	77	12	15.4	18.3***
	61	9	14.7	10.8**
	39	5	13.0	5.9+
	38	1	3.0	n.s.
	88	2	2.5	n.s.
	104	2	2.2	n.s.
	95	1	1.1	n.s.
	100	1	1.1	n.s.
23 fruits	1–133	0	0.00	
Matina	67	22	28.2	153.2***
	64	7	11.3	17.5***
	52	3	6.3	n.s.
	51	2	4.3	n.s.
	74	2	3.0	n.s.
	114	1	0.9	n.s.
30 fruits	2–159	0	0.0	
Ostravske Rane	23	6	23.7	162.1***
	83	3	4.0	12.8**
	120	1	0.9	n.s.
	188	1	0.6	n.s.
30 fruits	12–188	0	0.00	

[a]Test of individual fruits against average outcrossing per cultivar.
+, **, *** significant at p = .05, .001, .0001, respectively. n.s., not significant.

4 | DISCUSSION

In outdoor conditions, the productivity of tomato plants is strongly influenced by microclimatic conditions. The growing season is limited by the last frosts in spring and the first frosts in autumn or by terminal late blight infections. In this study, infections by *P. infestans* varied largely between environments and cultivars. The germination rate varied with climatic conditions and was also influenced by late blight fruit infection (data not shown). These factors resulted in differing numbers of pollination events evaluated for environments and cultivars (Tables 3 and 4).

4.1 | Effect of the cultivar

Very limited evidence was available on genotypic variation. Lesley (1924) observed largely differing outcrossing rates for the cultivars 'Magnus' (4.9%) and 'Dwarf Champion' (0.59%). The results presented here are in a similar range: no outcrossing was observed in 13 cultivar–location combinations in 2005 (Table 3); the highest value was 5.23% in 'Rosa Roma' 2006 (Table 4). Flower morphology can help to understand genotypic differences and to identify genotypes that may be prone to outcrossing. According to Lesley (1924), 'Magnus' had long styles, on average protruding 1.4 mm from the

TABLE 6 The potential to accept foreign pollen of six tomato cultivars at Schönhagen, Germany, 2005

Cultivar	Fruits	Potato-leaf seedlings	Cut-leaf seedlings	Outcrossing (%)
Galina Siberian Cherry	7	300	3	1.1
Quedlinburger Frühe Liebe	7	499	0	0.0
Rosa Roma	7	295	284	56.1
Matina	8	427	117	24.6
Ostravske Rane	8	599	11	2.1
Domaca Pfarrgarten	7	125	247	75.1

anther cone and 'Dwarf Champion' had short styles with the stigmata usually 1 mm inside the anther cone. A high outcrossing rate was observed in a genotype with protruding stigma in the Punjab (Singh et al., 2012) 9.3% in 25 m distance from a pollen donor and 6.6% at 50 m. In 'Rosa Roma', protruding stigmata were observed. Own observations indicate another floral anomaly: flowers of beefsteak or slicer tomatoes like 'Domaca Pfarrgarten' (defined by having multiple fruit chambers) frequently have more than the five sepals, petals and stamina typical for tomato flowers. An increased number of stamina lead to buds opening at an unusually early stage thus exposing the stigma. This may be the reason why outcrossing in 'Domaca Pfarrgarten' was relatively high. The two phenomena exposing the style correspond to the very high potential to accept foreign pollen observed in 'Domaca Pfarrgarten' and 'Rosa Roma' (Table 6).

4.2 | Effect of the environment

Year-to-year variation may be significant as shown by Richardson and Alvarez (1957) who observed 0.3% and 1.9% outcrossing in 2 years at the Chapingo Research Station in Mexico using a potato-leaf cultivar with the fitting name Hoja de Papa. Groenewegen, King, and George (1994) observed no significant differences between years, locations, and the distance to other crops. Outcrossing rates ranged from 0.0007% to 0.41% in commercial tomato fields in California where 18 m x 18 m blocks were established in 3 years in two fields. Annual variation observed in the present study exceeds the range in past experiments. In particular in 'Rosa Roma', strict autogamy was observed in 2005 and outcrossing exceeding 5% in 2006. Differences between the 2 years might have been caused by higher temperatures and more sunshine hours during most of the flowering time in 2006 (Table 2) favouring insect activity.

4.3 | Differences between individual flowers/fruits

In our experiment, the outcrossing rate of individual flowers varied largely (Table 5). In all three cultivars studied, fruits with zero, one or two seeds derived from outcrossing were present as well as fruits with many hybrid seeds and outcrossing rates in excess of 20%. It remains unclear to what extent wind pollination is active in tomato. Tomato pollen is not sticky (Gladis et al., 1996) and may be moved by wind, but inserted stigmata in mainly downward-facing flowers cannot easily be reached. An even distribution of pollen would be typical for wind pollination. Insect visits, however, usually occur in a more irregular pattern; insects working on individual flowers to gather pollen and nectar would explain many hybrid seeds in the resulting fruits. Outcrossing does happen when receptive stigmata are exposed and pollen from other plants is available. The former is reflected in the potential of flowers to accept foreign pollen (Table 6), and the latter is influenced by weather conditions and the abundance of pollinators and their interest in tomato pollen and nectar. The comparison of a high potential of flowers to accept foreign pollen to much lower spontaneous outcrossing rates is an indicator that tomato flowers were of very limited interest to pollinators.

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