Supplementary Materials

Effects of Few-Layer Graphene on the Sexual Reproduction of Seed Plants: An In Vivo Study with Cucurbita pepo L.

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Figure S1. (a) *Cucurbita pepo* L. stigmas treated without nanomaterials (CTRL); (b) with 1 mg of few-layer graphene (FLG); (c) with 1 mg of muscovite mica (MICA). Bar = 2 mm.
Figure S2. Physical–chemical characterization of few-layer graphene (FLG, left column) and muscovite (MICA, right column): (a) average Raman spectra; (b) X-ray powder diffraction; (c) thermogravimetric and elemental analysis; (d) energy dispersive X-ray (EDX) analysis; (e, f) lateral size distribution of sheets ($n > 80$); (g) representative TEM image of FLG; (h) representative TEM image of MICA.
Figure S3. Atomic force microscopy (AFM) characterization of few-layer graphene (FLG, left column) and muscovite (MICA, right column): (a) representative AFM images of FLG flakes; (b) representative AFM images of MICA nanocrystals; (c) height profile of (a); (d) height profile of (b); (e) thickness distribution of FLG flakes; (f) thickness distribution of MICA nanocrystals (n = 20 for (e) and (f)).
Table S1. Total reflection X-ray fluorescence (TXRF) elemental analysis of few-layer graphene (FLG).

| Element | Line | Concentration mg/L | Sigma/ mg/L | RSD/ % | LLD/ mg/L | Net Area | Background | Chi |
|---------|------|---------------------|-------------|--------|-----------|----------|------------|-----|
| Al      | K12  | 0.44                | 0.18        | 42.0   | 0.38      | 121      | 1224       | 1.44|
| Si      | K12  | 69.98               | 0.41        | 0.6    | 0.16      | 44845    | 1214       | 4.24|
| S       | K12  | 0.905               | 0.029       | 3.3    | 0.044     | 2130     | 1189       | 1.81|
| Cl      | K12  | 0.039               | 0.013       | 34.2   | 0.027     | 149      | 1225       | 0.88|
| K       | K12  | 0.089               | 0.007       | 7.3    | 0.012     | 755      | 1105       | 0.97|
| Ca      | K12  | 0.503               | 0.025       | 1.8    | 0.010     | 5154     | 1184       | 0.54|
| Ti      | K12  | 6.396               | 0.025       | 0.4    | 0.005     | 125219   | 1245       | 1.50|
| V (IS)  | K12  | 2.000               | 0.011       | 0.6    | 0.003     | 49353    | 795        | 1.66|
| Fe      | K12  | 0.019               | 0.001       | 5.0    | 0.001     | 894      | 523        | 0.86|
| Ni      | K12  | 0.014               | 0.001       | 4.7    | 0.001     | 966      | 497        | 0.99|
| Cu      | K12  | 0.017               | 0.001       | 3.8    | 0.001     | 1361     | 586        | 0.84|
| Zn      | K12  | 0.054               | 0.001       | 1.6    | 0.001     | 5071     | 465        | 1.38|
| Br      | K12  | 0.002               | 0.000       | 10.6   | 0.000     | 353      | 507        | 1.26|
| Sr      | K12  | Not det.            |             |        | 0.001     | 1        | 1353       | 3.78|
Table S2. Permutational multivariate analysis of variance (PERMANOVA) comparison of Cucurbita pepo L. pollen viability of untreated (CTRL) and few-layer graphene (FLG)- or muscovite mica (MICA)-treated samples at 2 and 0.5 mg per g (fresh weight) of pollen after 15 (T1), 45 (T2), 90 (T3), 180 (T4), and 360 (T5) minutes. Values are reported as mean ± s.d. N: Number of replicates per single treatment; Pseudo-F: Statistic computed for the single factor (for more details see text) by PERMANOVA; P(perm): Permutation p-value; statistically different groups (Monte Carlo post hoc test) at the same time point are marked with different letters [for P(perm) < 0.05].

| Treatment | Viability (%) | Pseudo-F | P(perm) |
|-----------|--------------|----------|---------|
|           | N  | T0   | T1   | T2   | T3   | T4   | T5   |         |
| at 2 mg g⁻¹ | 2.3219 | 0.1021 |
| CTRL      | 4  | 100 ± 8.1 | 80.6 ± 12.4 | a | 81.3 ± 8.2 | a | 81.4 ± 12.8 | a | 76.3 ± 13.4 | a | 74.9 ± 6.2 | a |
| FLG       | 4  | 72.2 ± 5.1 | a | 67.5 ± 12.3 | a | 59.0 ± 20.9 | a | 56.8 ± 15.9 | a | 60.1 ± 13.9 | a |
| MICA      | 4  | 73.4 ± 4.0 | a | 72.8 ± 11.7 | a | 75.5 ± 1.8 | a | 72.0 ± 12.9 | a | 57.6 ± 21.2 | a |
| at 0.5 mg g⁻¹ | 0.54628 | 0.6065 |
| CTRL      | 4  | 100 ± 8.5 | 81.5 ± 12.0 | a | 79.2 ± 13.4 | a | 73.1 ± 13.1 | a | 15.6 ± 15.5 | a | 74.3 ± 11.8 | a |
| FLG       | 4  | 74.9 ± 8.7 | a | 76.0 ± 7.9 | a | 71.0 ± 12.0 | a | 71.1 ± 16.3 | a | 57.9 ± 13.4 | a |
| MICA      | 3  | 84.9 ± 13.9 | a | 81.0 ± 12.7 | a | 74.3 ± 9.4 | a | 74.9 ± 14.3 | a | 71.1 ± 21.4 | a |
Table S3. Permutational multivariate analysis of variance (PERMANOVA) comparison of pollens detached (Detachment) and pollen germination percentage (Germination) on stigmas from pristine (CTRL) stigmatic surface of *Cucurbita pepo* or pretreated with 1 mg of few-layer graphene (FLG) and muscovite (MICA) for 3 h. Pollen detachment was evaluated after 40 min from pollination on the washing solutions derived from the application of the aniline blue staining protocol; after the same period, pollen germination was assessed on cross-sections of washed stigmas (for more details, see text). Values are reported as mean ± s.d.; N: Number of replicates per single treatment; Pseudo-F: Statistic computed for the single factor by PERMANOVA; P(perm): Permutation p-value; statistically different groups (Monte Carlo post hoc test) are marked with different letters [for P(perm) < 0.05].

| Treatment | Pseudo-F | P(perm) | Detachment   | Pseudo-F | P(perm) | Germination |
|-----------|----------|---------|--------------|----------|---------|-------------|
|           | 5.883    | 0.059   | N            | 28.21    | 0.001   | N           |
| CTRL      | 3        | 447 ± 170 | a            | 6        | 59.0 ± 4.51 | a          |
| FLG       | 3        | 1093 ± 207 | b            | 5        | 23.7 ± 5.24 | b          |
| MICA      | 3        | 924 ± 317  | ab           | 6        | 31.6 ± 12.3 | b          |
**Figure S4.** (a, b) SEM micrographs of stigmatic papillae of *Cucurbita pepo* female flowers treated for three hours without nanomaterials (CTRL); (c) with 1 mg of few-layer graphene (FLG); (d) with 1 mg of muscovite mica (MICA). Stigmatic papillae are indicated with arrows, germinating pollen grain with asterisk, nanomaterials with arrowheads. Bars = 100 µm.